

## Claremont Colleges Scholarship @ Claremont

---

CMC Senior Theses

CMC Student Scholarship

---

2011

# Preventing Mother-to-Child Transmission of Human Immunodeficiency Virus-1 (HIV-1): Effects of Intrapartum and Neonatal Single-Dose Nevirapine Prophylaxis and Subsequent HIV-1 Drug Resistance at Antiretroviral Treatment Initiation

Amanda L. Harmon  
*Claremont McKenna College*

---

### Recommended Citation

Harmon, Amanda L., "Preventing Mother-to-Child Transmission of Human Immunodeficiency Virus-1 (HIV-1): Effects of Intrapartum and Neonatal Single-Dose Nevirapine Prophylaxis and Subsequent HIV-1 Drug Resistance at Antiretroviral Treatment Initiation" (2011). *CMC Senior Theses*. Paper 305.  
[http://scholarship.claremont.edu/cmc\\_theses/305](http://scholarship.claremont.edu/cmc_theses/305)

This Open Access Senior Thesis is brought to you by Scholarship@Claremont. It has been accepted for inclusion in this collection by an authorized administrator. For more information, please contact [scholarship@cuc.claremont.edu](mailto:scholarship@cuc.claremont.edu).

**PREVENTING MOTHER-TO-CHILD TRANSMISSION OF HUMAN  
IMMUNODEFICIENCY VIRUS-1 (HIV-1): EFFECTS OF INTRAPARTUM AND  
NEONATAL SINGLE-DOSE NEVIRAPINE PROPHYLAXIS AND SUBSEQUENT  
HIV-1 DRUG RESISTANCE AT ANTIRETROVIRAL TREATMENT INITIATION**

Amanda L. Harmon

Submitted to the W.M. Keck Science Department of the Claremont Colleges  
in partial fulfillment of the requirements for the degree of Bachelor of Arts

SENIOR THESIS IN BIOLOGY  
CLAREMONT MCKENNA COLLEGE  
DECEMBER 2011









## **ABSTRACT**

The prevention of mother-to-child transmission is one of the most powerful tools in human immunodeficiency virus type 1 (HIV-1) prevention and has huge potential to improve both maternal and child health. In the absence of any preventative measures, infants born to and breastfed by their HIV-positive mothers have roughly a one-in-three chance of acquiring the infection themselves. HIV can be passed on from mother-to-child during pregnancy, during labor and delivery, and even after during breastfeeding.

Intrapartum and neonatal single-dose nevirapine (sd-NVP) is the foundation of preventing mother-to-child transmission in lower resource settings where it has been used alone or as part of combination regimens. Both its simplicity and its long plasma half-life contribute to the success of sd-NVP based therapy. However, sd-NVP frequently results in HIV-1 viral resistance in mothers and children who become HIV infected despite prophylaxis. Sd-NVP leads to the development of non-nucleoside reverse transcriptase inhibitors (NNRTI) drug resistance, compromising the success of treatment of mother and child with subsequent antiretroviral combinations. Resistance to NNRTIs is particularly worrisome in lower resource settings since many subsequent regimens for maternal and infant antiretroviral therapy include a NNRTI drug.



## **CONTENTS**

LIST OF TABLES	VII
LIST OF FIGURES	VII
ABBREVIATIONS AND ACRONYMS	XI
AN OVERVIEW OF HIV-AIDS	1
MOTHER-TO-CHILD TRANSMISSION	2
ANTI-HIV THERAPY	3
SEVERITY AND SCOPE OF THE PEDIATRIC HIV EPIDEMIC	5
DISPARITY BETWEEN PREVENTING MOTHER-TO-CHILD TRANSMISSION IN LOWER AND HIGHER RESOURCE SETTINGS	6
GLOBAL PREVENTION OF MOTHER-TO-CHILD TRANSMISSION: INADEQUATE PREVENTION EFFORTS	7
ELEMENTS OF SUCCESS: PREVENTING MOTHER-TO-CHILD TRANSMISSION	8
MATERNAL HEALTH INFLUENCES CHILD HEALTH OUTCOMES	11
ANTIRETROVIRAL PROPHYLAXIS IN REDUCING MOTHER-TO-CHILD TRANSMISSION OF HIV	13
ANTIRETROVIRAL REGIMENS IN LOWER RESOURCE SETTINGS	16
NEVIRAPINE	17
NEVIRAPINE: WHY SINGLE-DOSE?	18
IS SINGLE-DOSE NEVIRAPINE IDEAL?	20



PREGNANCY AND ANTIRETROVIRAL DRUG RESISTANCE	<b>21</b>
ANTIRETROVIRAL RESISTANCE FOLLOWING SHORT-COURSE PROPHYLAXIS	<b>21</b>
OVERVIEW OF ANTIRETROVIRAL RESISTANCE EMERGING FROM SINGLE-DOSE NEVIRAPINE	<b>22</b>
COMMON MUTATIONS FOLLOWING NEVIRAPINE TREATMENT	<b>24</b>
SAFETY AND EFFICACY OF NEVIRAPINE PROPHYLAXIS FOR REDUCING HIV DURING BREASTFEEDING	<b>25</b>
NEVIRAPINE-ASSOCIATED RESISTANCE MUTATIONS: IMPACT ON TREATMENT	<b>29</b>
HIV DRUG RESISTANCE AT ANTIRETROVIRAL TREATMENT INITIATION SUBSEQUENT TO SINGLE-DOSE NEVIRAPINE EXPOSURE	<b>31</b>
2010 WORLD HEALTH GUIDELINES FOR THE PREVENTION OF MOTHER-TO-CHILD TRANSMISSION	<b>33</b>
LOPINAVAR/RITONAVIR: ANOTHER OPTION FOR CHILDREN PREVIOUSLY EXPOSED TO NEVIRAPINE?	<b>36</b>
POSITIVE EFFECTS OF ANTIRETROVIRAL THERAPY PROGRAMS IN LOW RESOURCE SETTINGS	<b>39</b>
ELIMINATION OF MOTHER-TO-CHILD TRANSMISSION IS POSSIBLE	<b>42</b>
CONCLUSIONS	<b>44</b>
ACKNOWLEDGEMENTS	<b>47</b>
LITERATURE CITED	<b>49</b>





## LIST OF TABLES

<b>TABLE 1.</b>	<b>RELATIONSHIP BETWEEN THE MATERNAL PLASMA HIV RNA AND MTCT RATES</b>	<b>12</b>
<b>TABLE 2.</b>	<b>ARV MEDICATIONS APPROVED AND AVAILABLE FOR CHILDREN IN LOWER RESOURCE SETTINGS</b>	<b>36</b>

## LIST OF FIGURES

<b>FIGURE 1.</b>	<b>MTCT IS POSSIBLE DURING PREGNANCY, LABOR AND DELIVERY, AND THROUGHOUT BREAST FEEDING AT DIFFERENT PROPORTIONS</b>	<b>2</b>
<b>FIGURE 2.</b>	<b>MORE THAN TWO MILLION CHILDREN ESTIMATED TO BE LIVING WITH HIV GLOBALLY 1990-2008</b>	<b>5</b>
<b>FIGURE 3.</b>	<b>SUMMARY OF THE PACTG 076 STUDY DESIGN AND ITS RESULTS</b>	<b>6</b>
<b>FIGURE 4.</b>	<b>ESTIMATED NUMBER OF NEW PEDIATRIC INFECTIONS WITH AND WITHOUT ARV PROPHYLAXIS, GLOBAL LEVEL, 1996-2008</b>	<b>8</b>
<b>FIGURE 5.</b>	<b>TIMELINE OF MTCT AND DIFFERENT ARV INTERVENTIONS AND THEIR POINTS OF INTERACTION (ADAPTED FROM ICAP 2010).</b>	<b>14</b>
<b>FIGURE 6.</b>	<b>SUMMARY OF THE CHER STUDY DESIGN</b>	<b>15</b>
<b>FIGURE 7.</b>	<b>EARLY TREATMENT REDUCES THE RISK OF DEATH</b>	<b>15</b>
<b>FIGURE 8.</b>	<b>LOW UPTAKE OF HIV TESTING AND ARV PROPHYLAXIS IN LOWER RESOURCE COUNTRIES, 2004-2008</b>	<b>16</b>
<b>FIGURE 9.</b>	<b>CHEMICAL STRUCTURE OF NEVIRAPINE</b>	<b>17</b>
<b>FIGURE 10.</b>	<b>SUMMARY OF THE HIVNET 012 TRIAL DESIGN AND ITS RESULTS</b>	<b>19</b>

<b>FIGURE 11.</b> PLOT OF MULTIPLE STUDIES GROUPED ACCORDING TO WHETHER MOTHERS AND CHILDREN RECEIVED ONLY SD-NVP OR SD-NVP + ADDITIONAL ARVS AT 4-8 WEEKS POSTPARTUM	<b>23</b>
<b>FIGURE 12.</b> NEVIRAPINE ASSOCIATED MUTATIONS ON THE REVERSE TRANSCRIPTASE GENE	<b>24</b>
<b>FIGURE 13.</b> SWEN AND SD-NVP EXPOSED HIV+ INFANTS DIAGNOSED WITHIN THE FIRST SIX WEEKS OF LIFE OR AFTER	<b>27</b>
<b>FIGURE 14.</b> NEVIRAPINE RESISTANCE RESULTS FROM SWEN AND SD-NVP EXPOSED INFANTS	<b>28</b>
<b>FIGURE 15.</b> SUMMARY OF THE KIBS STUDY DESIGN AND ITS RESULTS	<b>29</b>
<b>FIGURE 16.</b> NNRTI RESISTANCE MUTATIONS AT ART INITIATION IN HIV+ CHILDREN WITH PRIOR EXPOSURE TO SD-NVP	<b>30</b>
<b>FIGURE 17.</b> TIME TO VIROLOGIC FAILURE IN INFANTS PREVIOUSLY EXPOSED TO SD-NVP	<b>31</b>
<b>FIGURE 18.</b> SUMMARY OF THE P1060 STUDY DESIGN	<b>32</b>
<b>FIGURE 19.</b> TIME TO PRIMARY END POINT OF VIROLOGIC FAILURE OR DISCONTINUATION OF TREATMENT GREATER IN NVP IN COMPARISON TO LVP/R ARM IN CHILDREN UNDER 12 MONTHS OF AGE	<b>33</b>
<b>FIGURE 20.</b> SUMMARY OF 2010 PMTCT WHO GUIDELINES FOR HIV+ MOTHERS IN LOWER-RESOURCE COUNTRIES	<b>34</b>
<b>FIGURE 21.</b> SUMMARY OF 2010 PMTCT WHO GUIDELINES FOR INFANTS BORN TO HIV+ MOTHERS IN LOWER-RESOURCE COUNTRIES	<b>35</b>
<b>FIGURE 22.</b> SUMMARY OF THE NEVEREST NVP RESISTANCE STUDY DESIGN	<b>37</b>
<b>FIGURE 23.</b> NEVEREST NVP RESISTANCE STUDY: SUSTAINED VIRAL SUPPRESSION ON CHILDREN IN THE NVP SWITCH GROUP IN COMPARISON TO THE CONTROL GROUP AT 52 WEEKS.	<b>38</b>
<b>FIGURE 24.</b> CHILDREN RECEIVING ART HAVE HIGH WEIGHT-FOR-AGE Z SCORES AND CD4+ PERCENTAGES, ZAMBIA	<b>39</b>
<b>FIGURE 25.</b> DECLINE IN EARLY LIFE MORTALITY WITH PMTCT ART SERVICES IN KWAZULU-NATAL, SOUTH AFRICA (ADAPTED FROM NDIRANGUA ET AL. 2010).	<b>40</b>
<b>FIGURE 26.</b> INCREASED USE OF MORE COMPLEX ARV REGIMENS IN ICAP-SUPPORTED PMTCT PROGRAMS 2007-2009 IN EIGHT DIFFERENT AFRICAN COUNTRIES	<b>41</b>
<b>FIGURE 27.</b> NEW HIV INFECTIONS IN CHILDREN AGES 0-14 THROUGH MTCT FOR DIFFERENT SCENARIOS 2009-2015	<b>43</b>





## ABBREVIATIONS AND ACRONYMS

<b>AIDS</b>	acquired immune deficiency syndrome
<b>ART</b>	antiretroviral therapy
<b>ARV</b>	antiretroviral
<b>AZT</b>	azidothymidine (also known as zidovudine)
<b>AZT+3CT</b>	combivir
<b>CDC</b>	Centers for Disease Control and Prevention
<b>CD4+ cells</b>	helper T lymphocytes
<b>CHER</b>	Children with HIV Early Antiretroviral Therapy study
<b>DSMB</b>	Data and Safety Monitoring Board
<b>d4T</b>	stavudine
<b>FDA</b>	U.S. Food and Drug Administration
<b>FI</b>	fusion inhibitor
<b>HAART</b>	highly active antiretroviral therapy
<b>HIV</b>	human immunodeficiency virus
<b>HIV-1</b>	HIV Type 1
<b>HIV-2</b>	HIV Type 2
<b>HIV+</b>	HIV positive
<b>HIV-</b>	HIV negative
<b>HIVNET</b>	HIV Network for Prevention Trials
<b>ICAP</b>	International Center for AIDS Care and Treatment Programs
<b>II</b>	integrase inhibitor
<b>IMPAACT</b>	International Maternal Pediatric Adolescent AIDS Clinical Trials
<b>LPV/r</b>	lopinavar/ritonavir



<b>MTCT</b>	mother-to-child transmission
<b>NFV</b>	nelfinavir
<b>NRTI</b>	nucleoside reverse transcriptase inhibitor
<b>NNRTI</b>	non-nucleoside reverse transcriptase inhibitor
<b>NVP</b>	nevirapine
<b>PI</b>	protease inhibitor
<b>PMTCT</b>	prevention of mother-to-child transmission
<b>sd</b>	single-dose
<b>sd-NVP</b>	single-dose nevirapine
<b>SWEN</b>	Six Week Extended-Dose Nevirapine (study)
<b>UNAIDS</b>	Joint United Nations Programme on HIV/AIDS
<b>UNICEF</b>	United Nations Children's Fund
<b>WHO</b>	World Health Organization
<b>ZDV</b>	zidovudine (also known as azidothymidine)
<b>3TC</b>	lamivudine



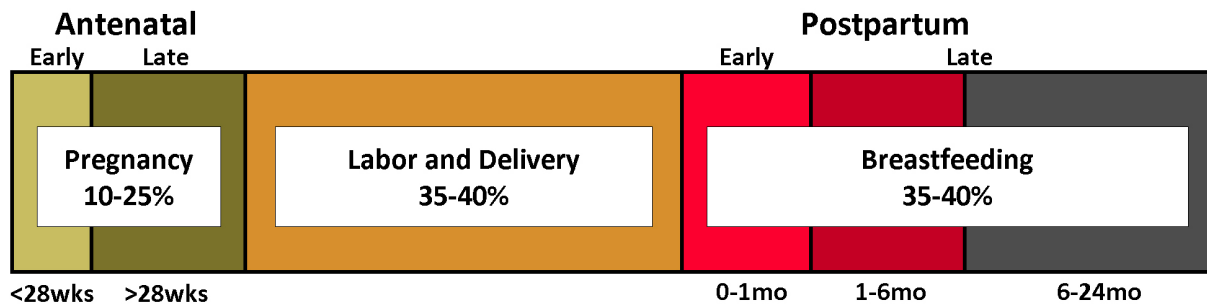


## **AN OVERVIEW OF HIV-AIDS**

HIV-1, the human immunodeficiency virus, is a retrovirus that can lead to acquired immune deficiency syndrome (AIDS). As a retrovirus, HIV stores its genetic information on single-stranded RNA instead of double-stranded DNA. After reverse transcription from the RNA template, HIV DNA enters the nuclei of the immune system's helper T lymphocytes (CD4+ cells) and is integrated into the cell's DNA. After infection, HIV then uses CD4+ cells as hosts and instructs each cell to make copies of the original virus. New virus particles are assembled and leave the cell to infect other CD4+ cells throughout the body. This entire process of HIV replication depletes the number of CD4+ cells, causing the immune system to become suppressed over time (Stine 2010). There is a less common, yet closely related strain of HIV called HIV-2 and unless otherwise stated, all subsequent mention of HIV is a reference to HIV-1.

HIV is the greatest health crisis the world faces today. An estimated 33 million people are living with HIV and an increasing number of women and children are being claimed by AIDS-related illnesses or death. Mother-to-child transmission (MTCT) is the most prevalent source of HIV infection in children. In 2000, the World Health Organization (WHO) issued recommendations on the use of antiretroviral drugs (ARVs) for preventing MTCT of HIV (WHO 2010). This thesis will discuss drug resistance induced by single-dose nevirapine (sd-NVP), a short-course ARV regimen used to prevent MTCT, which has been shown to not fully suppress the virus.

## MOTHER-TO-CHILD TRANSMISSION



**Figure 1.** MTCT is possible during pregnancy, labor and delivery, and throughout breast feeding at different proportions (Adapted from ICAP 2010).

Of the 1,200 new pediatric infections occurring daily, more than 90% are estimated to be attributed to mother-to-child transmission (MTCT) (UNAIDS 2009). MTCT occurs when an HIV-infected woman passes the virus to her baby. As depicted in Figure 1, this can happen during pregnancy, labor and delivery, or while breastfeeding. Not all infants born to women living with HIV will acquire HIV infection. Without any preventative measures, approximately 25-45% of children will acquire HIV from their untreated mother. The proportion of children who acquire infection varies by the timing of exposure. There is a disproportionately high percentage of infections, 35-40%, occurring during labor and delivery compared with pregnancy (Abrams 2010). During pregnancy, a woman can transmit the virus to her fetus in utero as the virus crosses over from the mother into the fetal bloodstream (Garcia et al. 1999 “Methods”). Although, it is more likely for newborns to acquire HIV during delivery by ingesting blood or other infected maternal fluids (Kuhn et al. 1994). If breastfed, the newborn may become infected from breast milk. The likelihood of infection during breastfeeding depends on the duration of breastfeeding and if the baby is exclusively fed breast milk or if there is mixed feeding (Abrams 2010). With cesarean

sections and the use of antiretroviral drugs, the risk of MTCT can be greatly reduced (WHO 2010).

## **ANTI-HIV THERAPY**

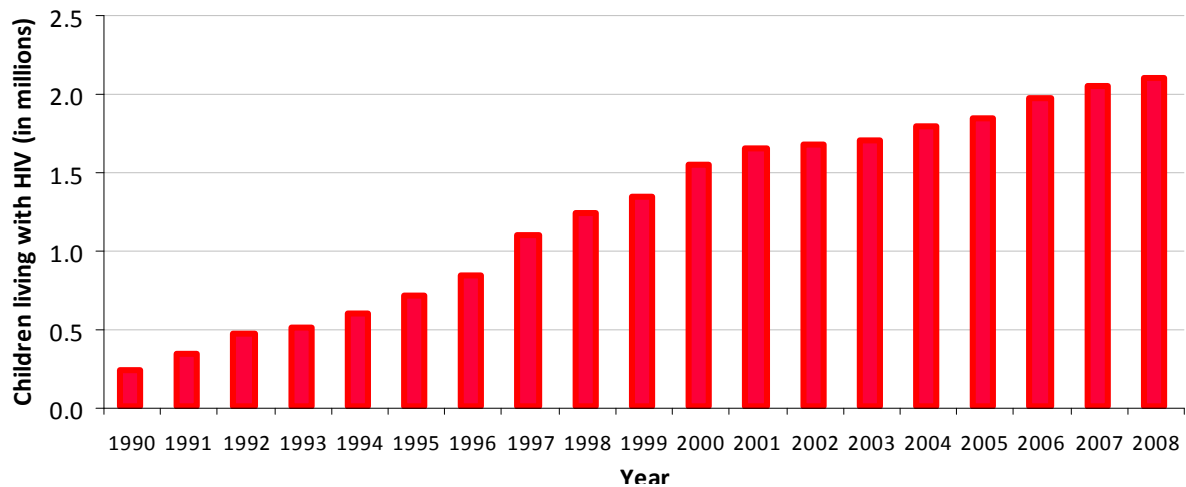
Although there is no cure for HIV, there are drugs called antiretrovirals (ARVs) that suppress the reproduction of the virus dramatically and help maintain the immune system. At present there are six different classes of antiretroviral HIV-drugs available.

1. Fusion inhibitors (FIs). FIs are also known as entry inhibitors and work outside the cell to prevent the first stage of HIV replication. They prevent HIV from entering the CD4+ cell by blocking fusion of HIV's outer membrane with the CD4+ cell membrane (Levy 2007).
2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs). NNRTIs bind noncompetitively to reverse transcriptase, which HIV uses to replicate, and inhibit the enzyme. This stops HIV from reproducing by preventing the conversion of RNA to DNA (Stine 2010).
3. Nucleoside reverse transcriptase inhibitors (NRTIs). NRTIs are sometimes referred to as "nukes" and like NNRTIs, interfere with the function of reverse transcriptase. NRTIs act as false substrates for reverse transcriptase, causing chain termination. This causes incomplete DNA synthesis and prevents HIV replication (Stine 2010).
4. Integrase inhibitors (IIs). IIs are a new drug class that blocks integrase, an enzyme that integrates HIV DNA into the nucleus of CD4+ cells (Stine 2010). The first II, raltegravir,

was approved by the U.S. Food and Drug Administration (FDA) in 2007 and can be administered to patients with NNRTI resistance (Hammer et al. 2008).

5. Protease inhibitors (PIs). PIs work at the last stage of HIV's replication cycle by targeting the HIV-1 protease enzyme which the virus uses to complete replication. By binding to HIV-1 protease, PIs prevent mature, infectious HIV from being successfully assembled and released from the infected CD4+ cell (Levy 2007).
6. CCR5 receptor antagonists. This is a new drug class that targets the CCR5 receptor, a CD4+ co-receptor which is involved in the HIV entry process. HIV binds to CCR5 receptor antagonists, blocking HIV from binding to CCR5 receptors on the cell and blocking entry of HIV (Stine 2010).

## SEVERITY AND SCOPE OF THE PEDIATRIC HIV EPIDEMIC



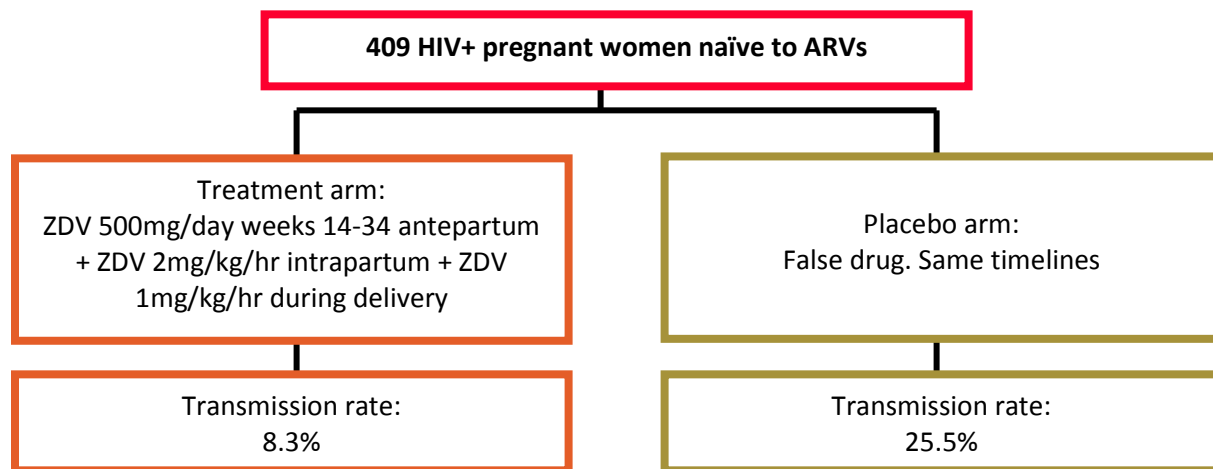
**Figure 2.** More than two million children estimated to be living with HIV globally 1990-2008 (Adapted from UNAIDS 2009).

Recent statistics provided by the Joint United Nations Programme on HIV/AIDS (UNAIDS) reveal the problematic and global immensity of MTCT. Of the estimated 33.4 million people living with HIV in 2008 alone, 2.1 million were children under 15 years of age (Figure 2). Even further, 430,000 of the 2.1 million children were newly infected with HIV in that year with the majority of them contracting the virus during the perinatal and breastfeeding period (UNAIDS 2009). Although this is a significant reduction from the estimated 500,000 in 2001, HIV continues to weigh heavily on child mortality in low resource countries especially those in sub-Saharan Africa. It is estimated that 85% of HIV positive (HIV+) children are living in sub-Saharan Africa where more than 90% of pediatric infections are attributed to MTCT (UNAIDS 2008). In contrast, new HIV infections in children are becoming increasingly rare in higher resource settings. In 2009, less than 1,000 children were estimated to have become infected in all of North America and Western Europe (UNAIDS 2010).



## DISPARITY BETWEEN PREVENTING MOTHER-TO-CHILD TRANSMISSION IN LOWER AND HIGHER RESOURCE SETTINGS

Although the pediatric HIV epidemic still rages overseas, it has been close to two decades since antiretroviral prophylaxis was proven effective in reducing MTCT. In 1994, the landmark study Pediatric AIDS Clinical Trials Group Protocol 076 (PACTG 076) demonstrated efficacy of the NRTI zidovudine (ZDV) to significantly reduce MTCT of HIV (Abrams 2010).



**Figure 3.** Summary of the PACTG 076 study design and its results (Adapted from Connor et al. 1994).

As shown in Figure 3, the PACTG 076 study placed 409 HIV+ pregnant women who were not previously exposed to ARVs in either a ZDV group or a placebo group. The mothers of the ZDV group were given 100mg of ZDV orally five times a day during weeks 14 to 34 antepartum. During intrapartum they were intravenously administered 2mg/kg body weight ZDV every hour and 1mg/kg every hour during delivery. Their infant(s) were given 2mg/kg body weight ZDV orally every six hours for the first six weeks of life. Results revealed an 8.3% rate of HIV-1 transmission in the ZDV group, a large drop in comparison to the 25.5% transmission rate of the placebo group. ZDV therapy also induced a 67.5%

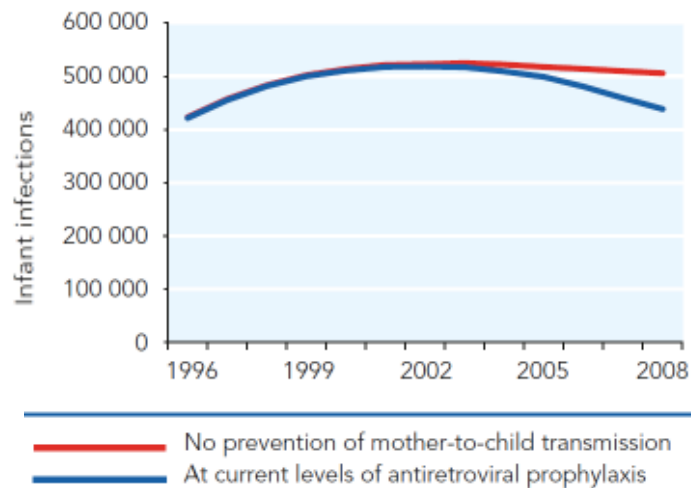
reduction in relative risk of HIV-1 transmission. A regimen based on this ZDV model soon became standard practice for PMTCT in high resource countries such as the United States (Connor et al. 1994). However, the complexity and expense of this approach, which entails both oral and intravenous dosing, made it impractical for use in most lower resource countries.

Despite the proven effectiveness of perinatal ZDV therapy based on the PACTG 076 study, there still remains a need for less complex and more cost effective regimens that could be used in both higher resource and lower resource settings. Today, the estimated MTCT rate is 30% in low resource countries. In stark contrast, MTCT rates are reported at 1-2% in most high resource settings around the world (Katz et al. 2008). MTCT has virtually been eliminated in high resource settings as a result of voluntary testing and counseling, widespread and routine access to ARV prophylaxis directed at suppressive therapy in pregnant women, safe delivery practices, and the ability and safe use of breast milk substitutes—all of which will be discussed further on (Townsend 2008). First, it must be understood why this disparity between low and high resource settings seems to plague global MTCT prevention strategies.

## **GLOBAL PREVENTION OF MOTHER-TO-CHILD TRANSMISSION: INADEQUATE PREVENTION EFFORTS**

It is clear that there has not been great success preventing pediatric infections in low resource, high HIV prevalence settings. Since prevention programs were put in place over a

decade ago, there has only been an estimated 200,000 averted infections and in 2008, only 70,000 pediatric infections were averted as seen in Figure 4 (UNAIDS 2009).



**Figure 4.** Estimated number of new pediatric infections with and without ARV prophylaxis, global level, 1996-2008 (UNAIDS 2009).

Turning to the key elements that guide the global strategy on PMTCT it is easy to see why this is such a difficult problem in lower resource settings.

## ELEMENTS OF SUCCESS: PREVENTING MOTHER-TO-CHILD TRANSMISSION

According to the United Nations, the four main elements of prevention that must be met are as follows:

1. Prevention of HIV in women of childbearing age.

In 2008, more than one million women were newly infected with HIV with an especially high risk among those entering reproductive years (UNAIDS 2009). Female youth are at a disproportionately high risk of acquiring HIV. There is a 3-5 fold higher prevalence of HIV among young girls in comparison to boys in high prevalence countries.

This can be seen in sub-Saharan Africa where 75% of youth living with HIV are females (WHO 2009).

For women living in high HIV-prevalent settings, the greatest risk of acquiring infection is through marriage or cohabitation. In a study done in Zambia and Rwanda, an estimated 55.1-92.7% of heterosexual transmission occurs within a marital or cohabiting relationship (Dunkle 2008). Most heterosexual HIV transmission for women takes place within marriage or cohabitation, a time in which most women start thinking about family. Therefore, counseling and testing for couples should be promoted to prevent any possible MTCT.

2. Prevention of unwanted pregnancies.

Worldwide, the prevention of unwanted pregnancies has not been met with success. There is an average of 80 million unintended pregnancies worldwide annually among women (Abrams 2010). Given this, it is not surprising that there is a very high unmet need for family planning among HIV-infected women. In lower resource countries, more than a quarter of women living with HIV report no desire for their current pregnancy or the wish to delay their next pregnancy by two years (UNAIDS 2010). In order to produce better outcomes for babies and their mothers and prevent new pediatric infections, there must be a strengthening of family planning services along with contraceptive use.

3. Prevention of transmission from an HIV+ woman to her child.

Most efforts for PMTCT depend on identifying HIV+ pregnant women and providing them with ARV prophylaxis treatment. In 2010, the proportion of pregnant

women in low and middle-income countries who received an HIV test reached 26%, up from 7% in 2005 (ICAP 2010). Although this shows progress, it is still a low figure. The development of these programs has been overwhelmed by the rapid and successful expansion of ARV treatment opportunities in low resource settings. However, one must note the disproportionately smaller number of pregnant women presently receiving antiretroviral therapy (ART) in comparison to the high number who are eligible for treatment. Since PMTCT programs are typically tacked on to already limited infrastructure for maternal-child health services, there has been a systematic failure to identify, prioritize, and treat pregnant women eligible for therapeutic ART. This is estimated to be about 20% of all pregnant women if using a CD4+ count of less than 200cells/ $\mu$ L. These women are at the highest risk for MTCT and for mortality (UNAIDS 2010).

In order to successfully achieve PMTCT, the infant must remain HIV negative (HIV-). It has been shown that an elective cesarean section reduces MTCT about 50% independent of ARV therapy (Garcia et al. 1999 “Mode”). This is difficult to achieve in settings with limited implementation of PMTCT programs. A limited infrastructure cannot provide a large number of antenatal care visits, institutional deliveries, or complex medical interventions. And even if there is a program in place, most programs still rely on short-course, monotherapy ART regimens that are of modest effectiveness and prone to antiretroviral resistance. Although short-course regimens are a relatively straightforward method of prevention, they only provide acute, episodic care rather

than recognizing the chronic nature and duration of exposure that occurs during pregnancy and breastfeeding (Abrams 2010).

As stated previously, the risk of MTCT extends throughout breastfeeding. In settings where breastfeeding is essential for child survival, milk substitutes are generally not safe or feasible. Therefore, there is still a focus on the unsuccessful infant feeding methods (Abrams 2010).

4. Providing care and treatment for HIV-infected mothers and their family.

For every mother living with HIV, a family is affected. If the disease kills a mother, it has the ability to fuel despair among her children and family. It has recently been found that keeping HIV+ adults in the family healthy reduces the risk of pediatric HIV and orphanhood. In their work in Uganda, Mermin and colleagues treated HIV+ adults with ART and cotrimoxazole, a sulfa drug that eliminates bacteria associated with common infections. They found an 81% reduction in death of uninfected children less than ten years old within the household as well as a startling 93% reduction in orphanhood (Mermin et al. 2008).

## **MATERNAL HEALTH INFLUENCES CHILD HEALTH OUTCOMES**

Over the last decade, scientists have identified two factors that have the biggest impact on transmission: the degree of advancement of maternal infection and ARV medications (Abrams 2010). It is clear that both these factors have become linked and this interconnectedness will be discussed in detail later.

Women with advanced HIV disease are at the greatest risk for their own disease progression and for transmission. In 1999, Garcia and colleagues identified the relationship between the risk of MTCT and the maternal viral load. As seen in Table 1, women with higher levels of plasma HIV RNA were associated with increasing rates of MTCT transmission. The risk was 0% among women with less than 1,000copies/mL. The highest rate of transmission was among women whose plasma HIV RNA levels exceeded 100,000copies/mL with 40.6% of non-breastfeeding babies acquiring HIV (Garcia et al. 1999 “Maternal”). Using the data found in the Garcia et al. study, statistically the mean maternal HIV-1 RNA viral load of transmission is 30,000copies/mL and the mean viral load of non-transmitters is 10,000copies/mL.

**Table 1.** Relationship between the maternal plasma HIV RNA and MTCT rates (Garcia et al. 1999 “Maternal”).

Maternal HIV RNA	N	Transmission N (%)
<1000	57	0 (0%)
1000-10,000	193	32 (16.5%)
10,001-50,000	183	39 (21.3%)
50,001-100,000	54	17 (30.9%)
>100,000	64	26 (40.6%)

Children born HIV+ to women with advanced HIV disease are more likely to show high viral loads during the first months of life and are at a higher risk of developing AIDS or dying (Shearer 1997). Even those born HIV- have a higher risk of death when born to mothers with advanced HIV. In a study done in Zimbabwe, it was found that uninfected infants born to infected mothers have at least twice the mortality rate of infants born to uninfected mothers (Marinda 2007).

## **ANTIRETROVIRAL PROPHYLAXIS IN REDUCING MOTHER-TO-CHILD TRANSMISSION OF HIV**

Antiretroviral drugs (ARVs) reduce perinatal transmission by several mechanisms, including lowering maternal antepartum viral load and providing the infant with pre- and post-exposure prophylaxis. A combination of antepartum, intrapartum, and infant ARV prophylaxis is recommended to prevent MTCT (“Panel on Treatment” 2011).

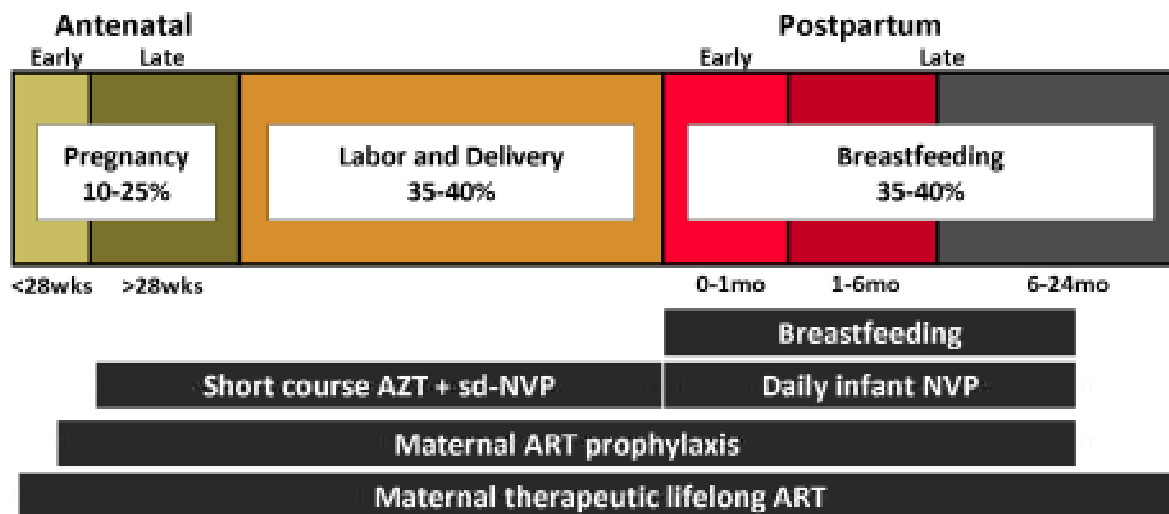
Lowering maternal viral load. Antenatal ARVs lessen the maternal viral load, which is particularly important in women with high viral loads. Even among women with HIV RNA plasma levels less than 1,000copies/mL, ARV drugs have been shown to reduce the risk of transmission (Ioannidis et al. 2001).

Providing infant with prophylaxis. An infant can become pre-exposed to ARVs when ARV drugs cross the placental barrier and create systemic drug levels in the fetus. This mechanism of protection is important during the infant’s passage through the birth canal, a time in which it is exposed to the virus in the mother’s genital tract. Infant post-exposure prophylaxis can be achieved by administering ARVs soon after birth. This intervention provides protection from prior exposure to the mother’s HIV virus that may have occurred through labor or passage through the birth canal (Garcia et al. 1999 “The Mode”).

The efficacy of antiretroviral drugs in preventing mother-to-child transmission of HIV is multifactorial and varies with the type of regimen used and the duration over which it is administered. Combination regimens include different types of antiretroviral drugs and are more efficacious than monotherapies. Monotherapies, discussed later in detail, are prone



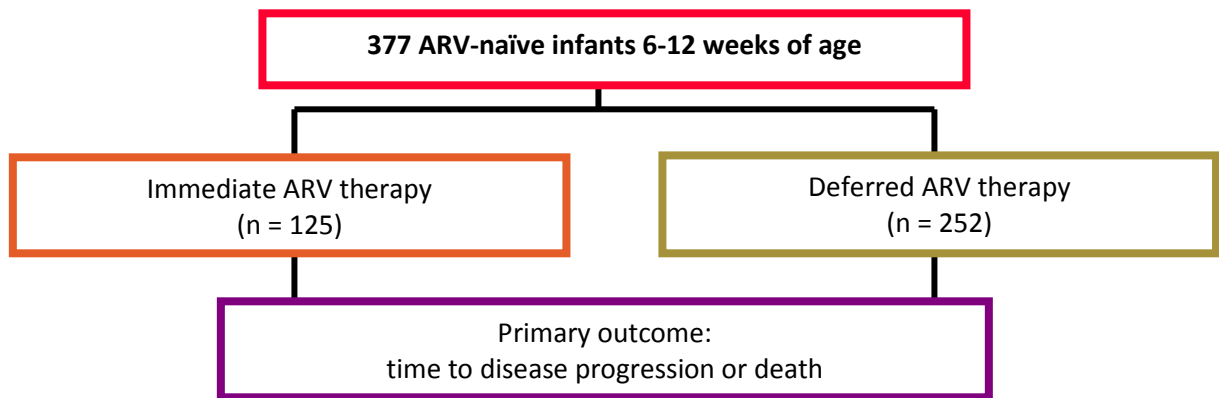
to antiretroviral resistance in the virus and may limit future therapeutic options when needed (“Panel on Treatment” 2011).



**Figure 5.** Timeline of MTCT and different ARV interventions and their points of interaction (Adapted from ICAP 2010).

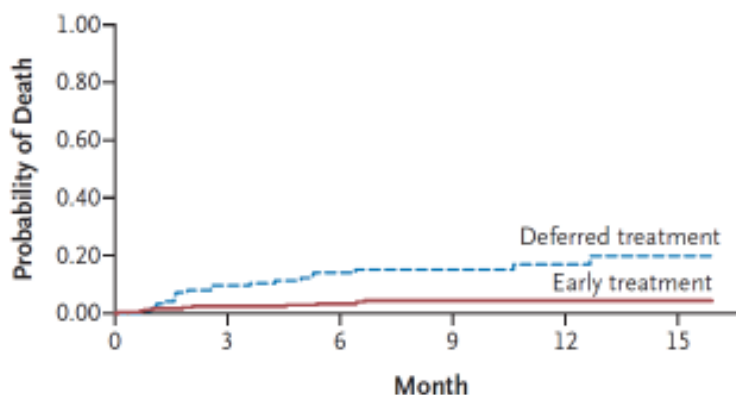
Antiretroviral medications given at any point along the timeline seen in Figure 5, can significantly decrease the risk of MTCT. In addition, ARVs given to the mother for their own health can result in maternal mortality, so ARVs should be given to the mother for her own survival. The timeline for MTCT in Figure 5 shows the different ARV interventions and their point of interaction. Single-dose nevirapine (sd-NVP), a NNRTI, is the most commonly used ARV prophylaxis. A single-dose is given to the laboring woman and her newborn infant. Sd-NVP interrupts transmission during labor and delivery and early postpartum period. If an additional short course of the NRTI azidothymidine (AZT, also known as zidovudine) is taken during late pregnancy through delivery along with sd-NVP, protection extends through late pregnancy. If a pregnant woman is treated for her own health, a maternal combination therapy or maternal ARV prophylaxis has the ability to protect both her and presumably her baby throughout the breastfeeding period. There has also been a shown

efficacy for protection during the postpartum period in breastfeeding infants who receive daily NVP (Figure 5, ICAP 2010).



**Figure 6.** Summary of the CHER study design (Adapted from Violari et al. 1994).

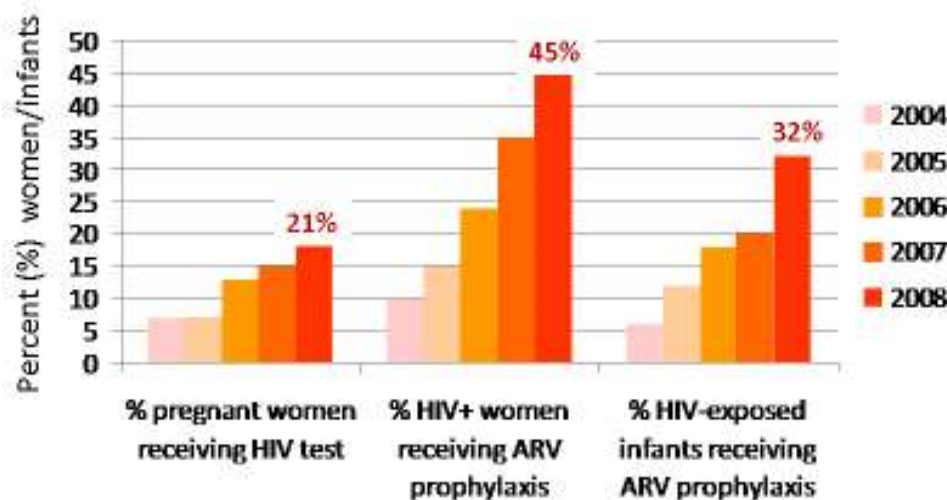
In the Children with HIV Early Antiretroviral Therapy (CHER) study, recent results definitively determined there is an advantage to starting children on treatment before to development of disease manifestations. In this landmark study, 377 infants six to twelve weeks of age who were not immune suppressed or symptomatic were randomized to start immediate treatment or deferred to ART when they met clinical criteria with a primary outcome of time to disease progression or death (Figure 6). It was concluded that an early HIV diagnosis and early ARV therapy during the first months of life reduced early infant mortality by 76% and HIV progression by 75% (Figure 7, Violari et al. 2008).



**Figure 7.** Early treatment reduces the risk of death (Violari et al. 2008).

## ANTIRETROVIRAL REGIMENS IN LOWER RESOURCE SETTINGS

Although antiretroviral regimens have been proven effective in the PMTCT, their scale up has not been effective in lower resource settings. Current statistics provided by United Nations Children's Fund (UNICEF) reveal the low uptake of HIV testing and ART for PMTCT in lower and middle resource settings. In their Fourth Stocktaking Report based on 2008 data, an estimated 21% of pregnant women living in lower resource countries received an HIV test. Yet, only 45% of those who tested positive received some form of ARV therapy or prophylaxis, and only 32% of exposed babies received some form of ARV prophylaxis (UNICEF 2009, Figure 8). And if an ARV is received, it is most commonly a single-dose of nevirapine, which will now be discussed.

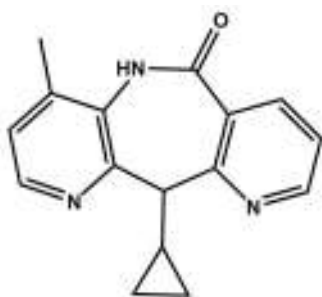


**Figure 8.** Low uptake of HIV testing and ARV prophylaxis in lower resource countries, 2004-2008 (Adapted from UNICEF 2009).

## NEVIRAPINE

Nevirapine (NVP), also marketed under the trade name Viramune, is a NNRTI produced by the U.S. manufacturer Boehringer Ingelheim Pharmaceuticals Inc. NVP was first approved by the U.S. Food and Drug Administration (FDA) in 1996 for use in combination therapy and in 1998 for pediatric use (Menéndez-Arias et al. 2011). Approval for combination therapy was based on a 1996 study done by D'Aquila and colleagues that showed that adding NVP to two NRTIs, zidovudine and didanosine, was more effective in increasing CD4+ counts and decreasing HIV viral load than zidovudine and didanosine alone (D'Aquila et al. 1996). NVP is currently the only NNRTI drug with pediatric drug formulation and neonatal dosing ("Panel on Treatment" 2011).

Structure. The chemical name of nevirapine is 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one and the molecular formula is  $C_{15}H_{14}N_4O$ . Its chemical structure can be seen in Figure 9 (Campiani et al. 2002).



**Figure 9.** Chemical structure of nevirapine (Adapted from Menéndez-Arias et al. 2011).

Mode of action. As a NNRTI, nevirapine is a potent noncompetitive inhibitor of the reverse transcriptase enzyme, preventing HIV's viral RNA from being transcribed into DNA. NVP does not bind at the enzyme's active site like NRTIs, but allosterically at the hydrophobic NNRTI pocket, a site away from the active site (Patel and Benfield 1996). NVP

selectively inhibits HIV-1, but not HIV Type 2 (HIV-2) since the NNRTI pocket on HIV-2's reverse transcriptase enzyme has a different structure and has a higher than 8,000 fold selectivity for infected than uninfected cells (Ren et al. 2002).

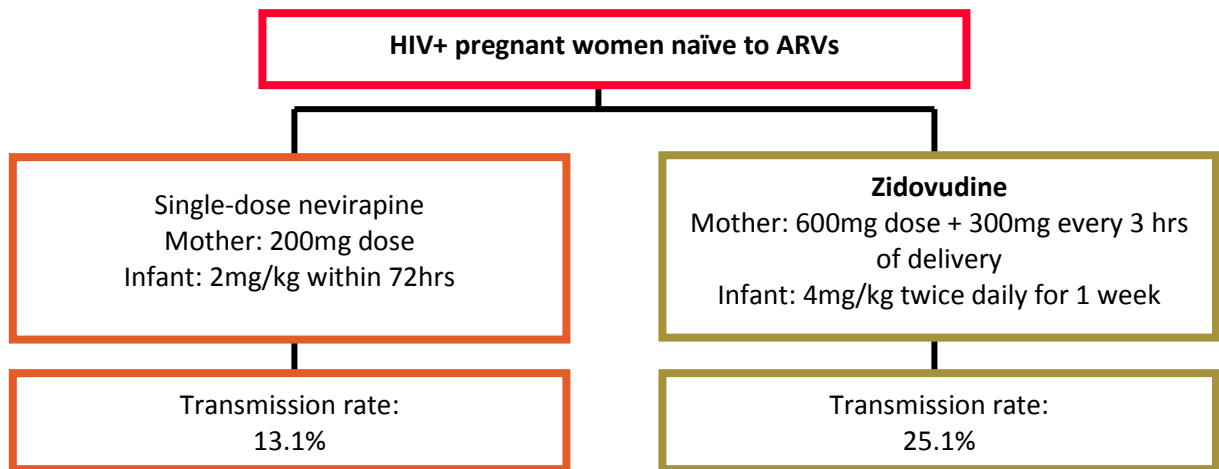
Dosage. Nevirapine comes in a tablet and a liquid oral suspension. It has been found that higher concentrations of NVP (2.5-10mg/L) work best and can completely suppress viral replication in cell cultures and protect uninfected cells (Patel and Benfield 1996).

Use in combination therapy. Addition of nevirapine to existing ART therapy produces a more rapid and sustained immunological and virological response. Nevirapine shows a higher synergistic inhibitory activity in combination with NRTIs. Due to the same molecular target, NNRTIs and NRTIs complement each other in interfering with viral replication even though they have different mechanisms of actions (Campiani et al. 2002). This was seen in the D'Aquila et al. study that NVP's FDA approval was based on.

So, if nevirapine works best in combination with NNRTIs, why do lower resource countries rely on a single-dose of NVP to prevent mother-to-child transmission?

## **NEVIRAPINE: WHY SINGLE-DOSE?**

HIVNET 012 trial. The simplest of all PMTCT drug regimens was tested in the HIV Network for Prevention Trials (HIVNET) 012 study, which took place in Uganda between 1997 and 1999. This randomized clinical trial evaluated a simple ARV drug regimen to prevent the transmission of HIV-1 from an infected mother to her child in a breastfeeding population.



**Figure 10.** Summary of the HIVNET 012 trial design and its results (Adapted from Guay et al. 1999).

As seen in Figure 10, the experimental drug regimen consisted of a single oral dose of 200mg nevirapine (sd-NVP) given at the onset of labor to HIV-infected pregnant women along with a single-dose of nevirapine (2mg/kg) given to the baby within 72 hours of life. The comparison group was given a zidovudine (ZDV) regimen consisting of a maternal oral dose of 600mg along with 300mg every three hours of labor, in addition to a 4mg/kg ZDV dose twice daily for the baby's first week of life. ZDV was previously shown effective in Conner et al.'s 1994 study. The HIVNET 012 study showed that the NVP regimen reduced MTCT risk by 47% at 14 to 16 weeks compared to the ZDV regimen. At that age, the overall transmission rate observed was 13.1% for the NVP group versus 25.1% for the ZDV group. The transmission rate for the ZDV group was similar to those observed in the placebo groups of other randomized clinical trials conducted in breastfeeding populations (Guay et al. 1999).

With its findings that a single-dose of NVP given to the mother at the onset of labor and to the baby after delivery can halve the rate of HIV transmission, the HIVNET 012 study jumpstarted the implementation of sd-NVP regimens in low resource settings with high HIV

prevalence. Coupled with rapid testing, women attending antenatal clinics could now be counseled, tested, and given a single pill that could save the life of their child.

An open access program by Boehringer Ingelheim Pharmaceuticals Inc., the U.S. manufacturer of NVP, and their local partners quickly facilitated the rapid expansion of PMTCT programs using sd-NVP throughout the world (Abrams 2010).

### **IS SINGLE-DOSE NEVIRAPINE IDEAL?**

Without a doubt, the HIVNET 012 study had and still has major implications for the control of HIV transmission in many developing countries and has led to critical decisions in the international health care community. The ease of administration and low cost of sd-NVP makes it ideal for monotherapy for prophylaxis of perinatal transmission, especially in settings where there are less resources and not enough antenatal care (Abrams 2010).

A study done by Marseille and colleagues in 1999 proposed the universal administration of sd-NVP to all pregnant women at the time of labor and delivery and to their newborns in settings with high HIV prevalence, regardless of HIV status (Marseille et al. 1999). This proposal was based on the cost effectiveness (about \$2/dose) and simplicity of sd-NVP along with taking into account the technological difficulties of administering HIV testing in pregnant women in these countries, the lack of prenatal care, and the relative safety of sd-NVP for mothers and infants (Peters et al. 1999).

This proposal, however, has led to opposition from those who question the risk of drug resistance caused by repeated exposure to nevirapine (during repeated pregnancies)

and who point out that \$4 is close to the annual health care expenditure per capita in some African countries that are severely afflicted by HIV infection and AIDS.

## **PREGNANCY AND ANTIRETROVIRAL DRUG RESISTANCE**

The development of ARV drug resistance is one of the major factors leading to therapeutic failure in HIV-infected individuals. In pregnant mothers, there are specific concerns that differ from the nonpregnant population. A pre-existing drug resistance to a drug used in an ARV regimen can diminish the regimen's efficacy for PMTCT. If a mother develops resistance to drugs used during pregnancy to stop MTCT, her future options may be limited. In addition, infant treatment options may also become limited if the mother develops a resistant virus that is then transmitted to her fetus ("Panel on Treatment" 2011).

Several factors unique to pregnancy may increase the risk of developing resistance. If drugs with significantly different half-lives such as the combination of NVP and two NRTIs are included in the regimen and the mother discontinues ARV therapy after delivery, there is an increased chance of NNRTI resistance due to the persistent subtherapeutic drug levels. Nausea and vomiting associated with early pregnancy may also compromise adherence and increase the risk of resistance (Lockman et al. 2007).

## **ANTIRETROVIRAL RESISTANCE FOLLOWING SHORT-COURSE PROPHYLAXIS**

Viral resistance may emerge during ARV treatment and occurs frequently with single-drug regimens and even more frequently with single-dose regimens. Viral resistance



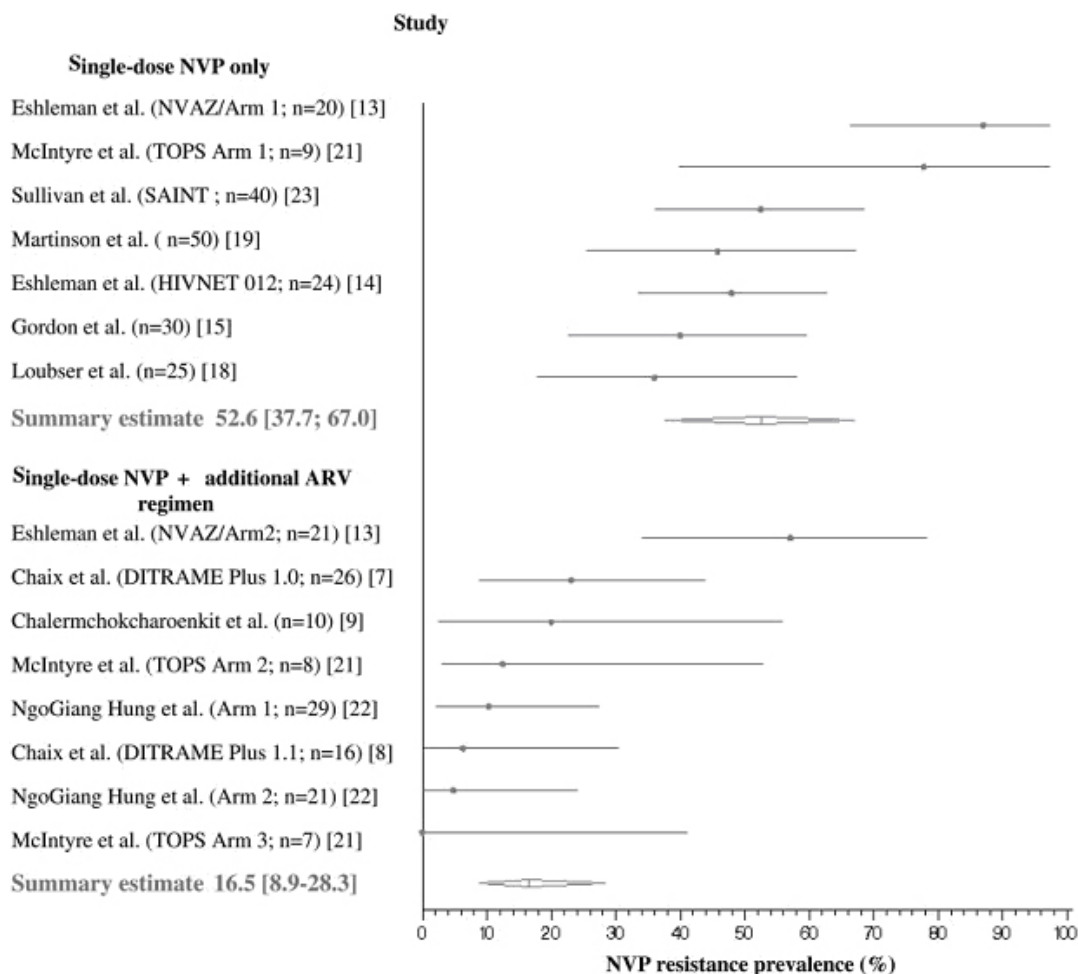
is a potential problem for women after short-term exposure to ARV drugs to prevent MTCT and for infants who become infected despite ARV prophylaxis.

Even before ARV drugs are administered, HIV that contains mutations associated with viral drug resistance is present at low levels not detectable using standard resistance assays. Pre-existing resistant viral populations may be selected for or new mutations may develop with any ARV drug or drug regimen that does not fully suppress viral replication. Most studies have also reported that a high maternal plasma viral load or a low CD4+ count are associated with an increased risk of resistance to any ARV drug. NNRTIs such as NVP are drugs for which a single mutation leads to high-level resistance, whereas most NRTI drugs require multiple sequential mutations to confer resistance (Mofenson et al. 2002).

## **OVERVIEW OF ANTIRETROVIRAL RESISTANCE EMERGING FROM SINGLE-DOSE NEVIRAPINE**

Development of resistance associated with short-term use of ARV drugs for PMTCT is most common with sd-NVP. Over the last decade there has been a great deal of work looking at the implications of NVP resistance mutations in mothers children who fail sd-NVP PMTCT prophylaxis. Arrivé and colleagues published a meta-analysis of summarized data looking at prevalence of NVP resistance mutations in the plasma of the mothers and infants at four to eight weeks postpartum after sd-NVP use for PMTCT. The pooled estimates of NVP resistance prevalence were found to be 35.7% in the mothers and 52.6% in the children following sd-NVP (Figure 8). However, the administration of postpartum ARVs to the mother can significantly reduce the frequency of detection of NVP-resistant strains. As

seen in Figure 11, the prevalence of NVP resistance can be significantly reduced to 4.5% in the mother and 16.5% in the child by adding short-course postpartum ARV therapy to standard sd-NVP (Arrivé et al. 2007). It is important to remember that NVP resistance is also associated with resistance to other NNRTIs due to the location of mutations on the reverse transcriptase gene.



**Figure 11.** Plot of multiple studies grouped according to whether mothers and children received only sd-NVP or sd-NVP + additional ARVs at 4-8 weeks postpartum (Arrivé et al. 2007).

Resistance to NVP develops rapidly if viral replication is not completely suppressed. Because of NVP's long half-life, the drug can be detected in plasma up to three weeks after administration of a single-dose during labor and delivery (Cressey et al. 2005). This long

half-life creates a long period of persistent subtherapeutic drug levels that cause a predisposition to the development of resistant strains of HIV (Arrivé et al. 2007). Viral strains resistant to NVP were detected at six weeks postpartum in 19% of ARV-naïve women of the HIVNET 012 trial and in 15% of women on additional ARV therapy who received sd-NVP during labor (Cunningham et al. 2002, Eshleman et al. 2001). The use of sd-NVP is likely to result in HIV viral resistance which could lead to resistance of subsequent ARV treatment combinations.

## COMMON MUTATIONS FOLLOWING NEVIRAPINE TREATMENT

<b>Amino acid, wild-type</b>	<b>L</b>	<b>K</b>	<b>V</b>	<b>V</b>	<b>Y</b>	<b>Y</b>	<b>G</b>
<b>Amino acid position</b>	<b>100</b>	<b>103</b>	<b>106</b>	<b>108</b>	<b>181</b>	<b>188</b>	<b>190</b>
<b>Amino acid, substitution conferring resistance</b>	<b>I</b>	<b>N</b>	<b>A</b> <b>M</b>	<b>I</b>	<b>C</b> <b>I</b>	<b>C</b> <b>L</b> <b>H</b>	<b>A</b>

**Amino acid abbreviations:** A, alanine; C, cysteine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; V, valine; Y, tyrosine

**Figure 12.** Nevirapine associated mutations on the reverse transcriptase gene (Adapted from International AIDS Society-USA 2006).

With nevirapine's low genetic barrier for resistance, single-nucleotide changes in the viral genome can cause high-level resistance to NNRTIs. The most prominent mutations found after exposure to NVP treatment are the Y181C mutation and the K103N mutation, correlating with amino acid changes in the hydrophobic pocket where NVP usually binds (Figure 12). As stated previously, NNRTIs exert their antiviral effect against HIV by binding to reverse transcriptase in a hydrophobic pocket located next to the active site of the enzyme that blocks the process of DNA polymerization, causing a conformational change in this enzyme (Patel and Benfield 1996). The region of the hydrophobic pocket that NNRTIs

bind predominantly involves amino acid codons 98-108 and 179-190. Since all NNRTIs bind within the same pocket, a mutation observed with one NNRTI is usually observed with all NNRTIs. Therefore, viral strains which are NVP resistant are also usually resistant to other NNRTIs (Conway et al. 2001, Deeks 2006). Resistance to NNRTIs occurs as a result of mutations that inhibit effective binding of the NNRTI, allowing DNA polymerization to proceed in an unrestricted manner (Deeks 2006).

Furthermore, the predominant NVP resistance mutations appear at different time points. In mothers, the Y181C mutation is predominant one week postpartum and the K103N is predominant six to eight weeks postpartum. However, most infants with NVP resistance were noted to be infected at birth, suggesting that the resistance mutations were not being passed on from the mother, but instead when their actively replicating virus was exposed to NVP (WHO 2010). Therefore, the transmission of resistant viral strains to infants is not associated with an increased risk of MTCT.

## **SAFETY AND EFFICACY OF NEVIRAPINE PROPHYLAXIS FOR REDUCING HIV DURING BREASTFEEDING**

HIV transmission during breastfeeding has also reduced the overall effectiveness of efforts to prevent MTCT. Multiple studies have found multiclass drug resistance in breastfeeding infants who became infected despite NVP prophylaxis. The overall risk of HIV transmission through breastfeeding is around 35-40% during the first two years of life, with the greatest risk by the first six to fourteen weeks of life, a time in which about 60-70% of breast milk transmission occurs (ICAP 2010). Despite this risk, WHO recommends that HIV-

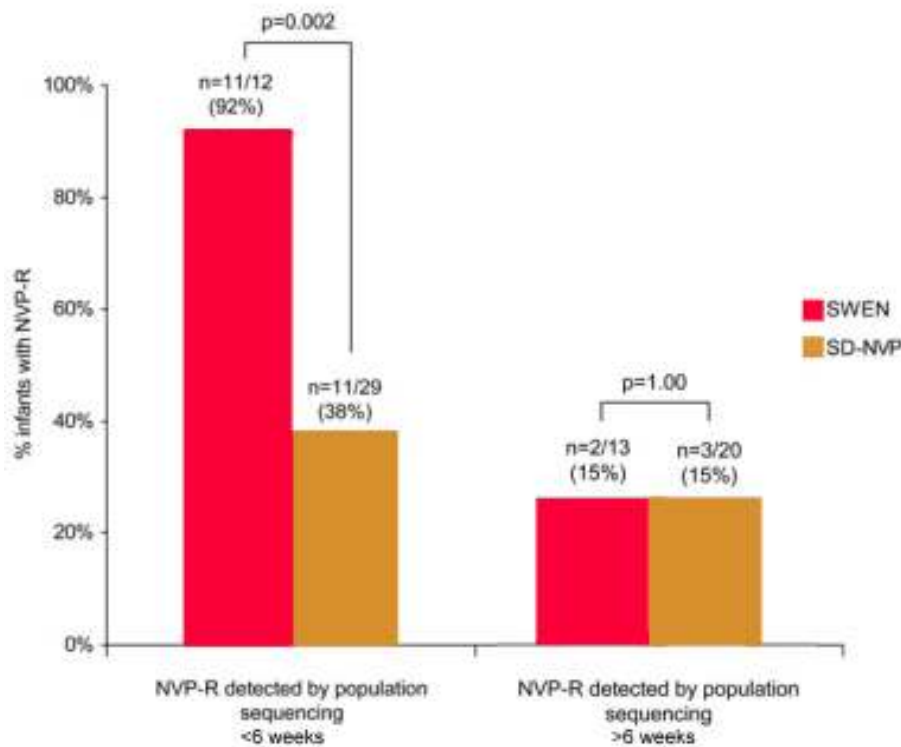
infected mothers living in lower resource countries exclusively breastfeed for at least six months to improve infant survival (WHO 2010).

Resistance mutations have been found in maternal plasma and breast milk after sd-NVP exposure. In a study of twenty women from Zimbabwe, Lee and colleagues looked at paired specimens of plasma and breast milk. They found that 50% of the plasma samples and 67% of the breast milk samples had detectable NNRTI mutations, predominantly the K103N mutation (Lee et al. 2005). This observation of drug-resistance in sd-NVP exposed women who breastfeed created much concern and led to trials investigating the use of daily NVP to preserve safe breastfeeding among HIV infected women.

To allow for breastfeeding, yet reduce transmission, NVP dosages were extended for up to six weeks of age in the “Six Week Extended-dose NVP” (SWEN) trial. The SWEN trial compared postnatal infection in breastfeeding infants in Uganda, Ethiopia, and India who received sd-NVP or six weeks of daily NVP. In infants uninfected at birth, MTCT at six weeks was 5.3% in the sd-NVP arm versus 2.5% in the extended NVP arm. At six months, MTCT was 9.0% in the sd-NVP arm versus 6.9% in the extended NVP arm (Moorthy et al. 2009). Extended NVP allows for daily NVP prophylaxis for infants breastfeeding and lowers the rate of HIV infection in comparison to sd-NVP.

Although the use of the SWEN regimen looks promising at preventing breast milk HIV transmission, a study completed by Moorthy and colleagues on the Indian arm of the SWEN trial showed that the SWEN regimen carries a high likelihood of NVP resistance if the infant becomes infected within the first six weeks of life. As demonstrated in Figure 13, of the infants diagnosed with HIV by six weeks of age, the SWEN-exposed infants had a

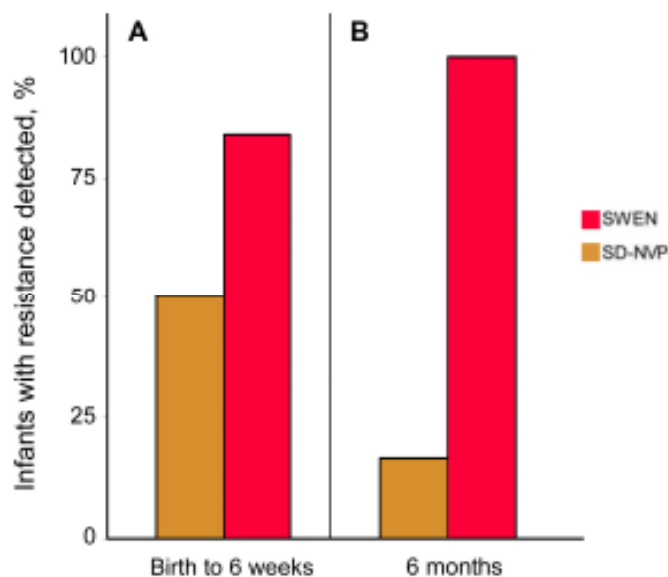
significantly higher prevalence of NVP resistance at 92% than those who received sd-NVP, of which only 38% developed NVP resistance when detected by standard population sequencing. After six weeks of age, the prevalence of NVP resistance did not differ among SWEN or sd-NVP exposed infants who became infected during breastfeeding (Figure 13, Moorthy et al. 2009). As with sd-NVP, the value of preventing HIV infection in a large number of infants should be considered alongside the high risk of resistance associated with extended NVP prophylaxis.



**Figure 13.** SWEN and sd-NVP exposed HIV+ infants diagnosed within the first six weeks of life or after (Adapted from Moorthy et al. 2009).

Similar findings to Moorthy et al. were found by Church and colleagues when they evaluated NVP resistance in the Ugandan SWEN cohort. At six weeks, 84% of the breastfeeding infants had NVP resistance in comparison to 50% who were only given sd-NVP (Figure 14A). This is a 1.7 fold greater risk of resistance in SWEN group compared with

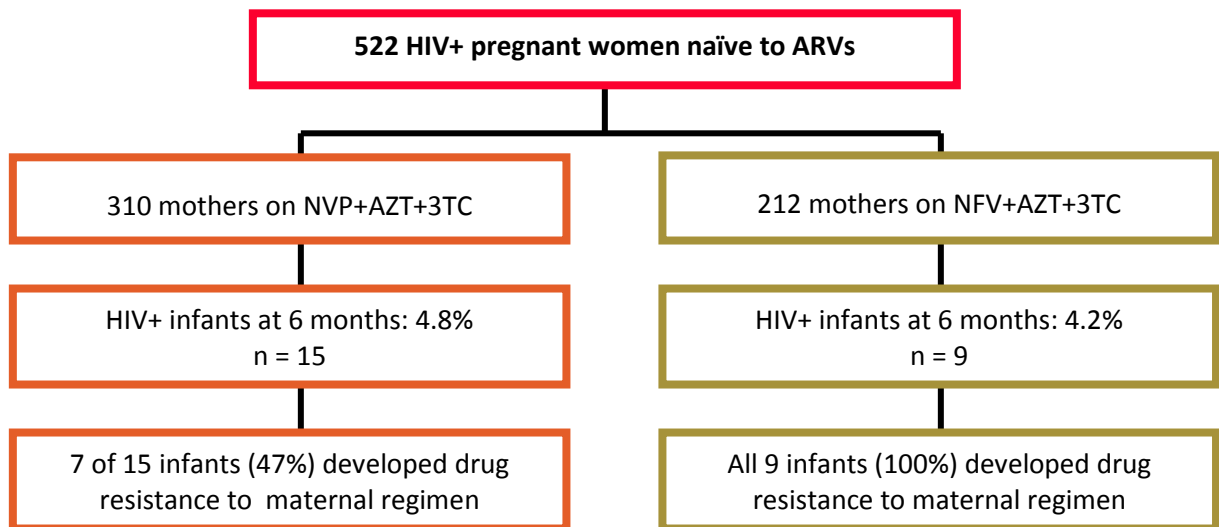
sd-NVP when tested at 6 weeks of age. When a small group of these children were retested a six months, 100% of 7 children in the SWEN group continued to have detectable NVP resistance whereas only 1 of the 6 children, or 17%, in the sd-NVP group had detectable resistance (Figure 14B, Church et al. 2008). These findings suggest that resistance is greater in children with longer duration of exposure.



**Figure 14.** Nevirapine resistance results from SWEN and sd-NVP exposed infants. **A**, percentage of infants with NVP resistance within first six weeks of life. **B**, percentage of infants who had NVP resistance mutations at six weeks that were retested at six months (Adapted from Church et al. 2008).

Resistance in breastfeeding HIV-infected infants of mothers on ART has also been demonstrated by the Kisumu breastfeeding study (KiBS) completed in Kenya. In the KiBS, pregnant women received either NVP or the protease inhibitor nelfinavir (NFV) along with the nucleoside reverse transcriptase inhibitor combivir (AZT+3CT) from 34 weeks gestation through 6 months postpartum. As seen in Figure 15, by six months of age, 24 infants were HIV infected. Of the 24 infants, 9 were born to mothers on the NFV regimen and 15 were

born to mothers on the NVP regimen. Resistance was detected among 9/9 (100%) of the NFV exposed infants and in 7/15 (47%) of the NVP exposed infants (Zeh et al. 2011)



**Figure 15.** Summary of the KiBS study design and its results (Adapted from Zeh et al. 2011).

Mirochnick and colleagues decided to evaluate the serum concentrations of NVP and 3TC of the uninfected infants of mothers on antiretroviral therapy in the KiBS. The median concentrations in breastmilk of NVP and 3TC were 1,214ng/mL and 4,546ng/mL in comparison to zidovudine's 14ng/mL (Mirochnick et al. 2009). These biologically significant concentrations of both NVP and 3TC in the infants demonstrate that NVP and 3TC can be transferred in breast milk.

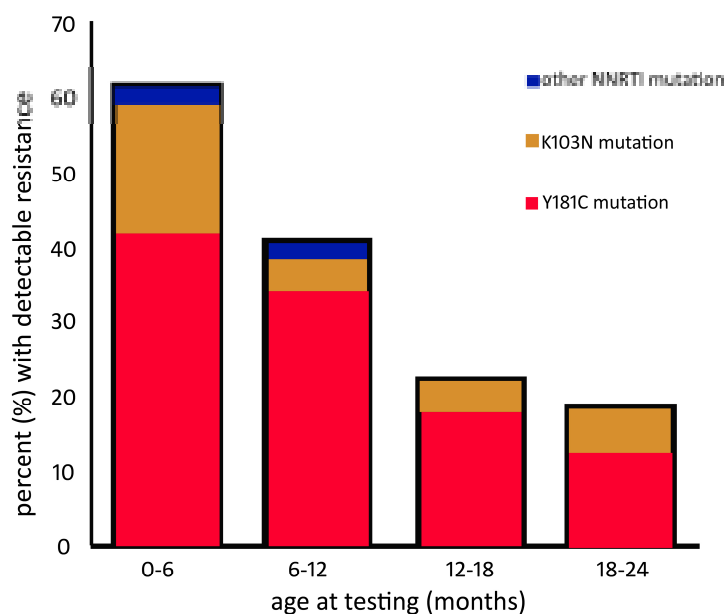
## NEVIRAPINE-ASSOCIATED RESISTANCE MUTATIONS: IMPACT ON TREATMENT

It is now well established that sd-NVP use and NVP use in general for PMTCT can cause viral mutations associated with NNRTI resistance. Infants who fail prophylaxis and acquire infection despite NVP exposure tend to develop resistance. So what does this all mean for the child with HIV infection presenting for treatment? The small numbers of



children infected in previous perinatal studies make it hard to determine especially when most have been studied only six to eight weeks after exposure.

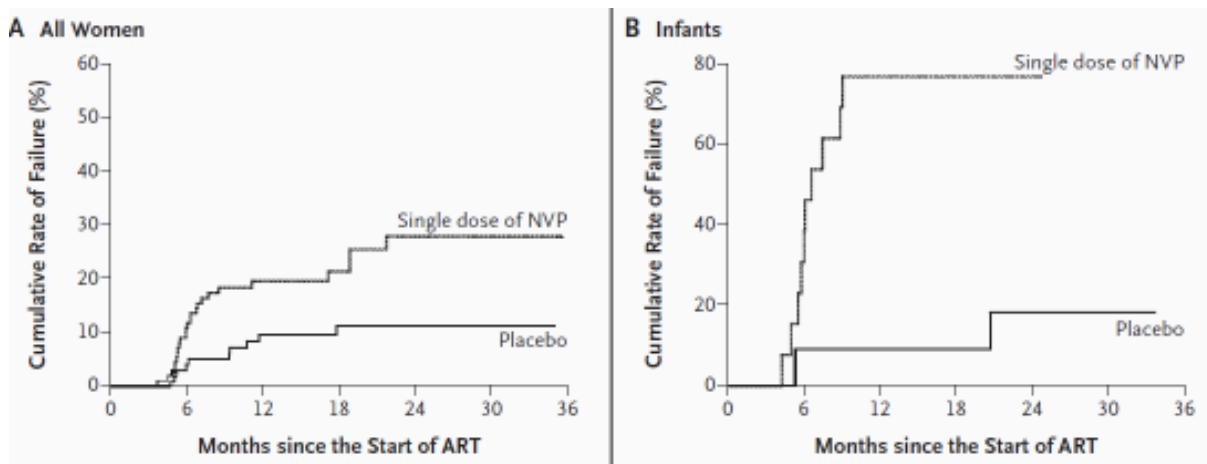
In the Nevirapine Resistance Study (NEVEREST), sd-NVP exposed children who were eligible for ART were started on a combination of LPV/r+AZT+3TC therapy. Yet prior to initiation of treatment, resistance testing was done for 257 symptomatic children between the ages of six months and two years. Overall, 27% of the children had detectable NNRTI major mutations using standard population sequencing. The Y181C mutation was found in 21% of the children and the K103N mutation was found in 5% of the children. Using allele specific PCR testing for low level frequencies, an additional 13% of Y181C mutations and 9% K103N mutations were detected. As seen in Figure 16, young children are at a much higher risk for any NNRTI mutation in comparison to older children (Hunt et al. 2009). This suggesting a fading of resistance over time, which may allows for ARV treatment subsequent to sd-NVP exposure.



**Figure 16.** NNRTI resistance mutations at ART initiation in HIV+ children with prior exposure to sd-NVP (Adapted from Hunt et al. 2009).

## HIV DRUG RESISTANCE AT ANTIRETROVIRAL TREATMENT INITIATION SUBSEQUENT TO SINGLE-DOSE NEVIRAPINE EXPOSURE

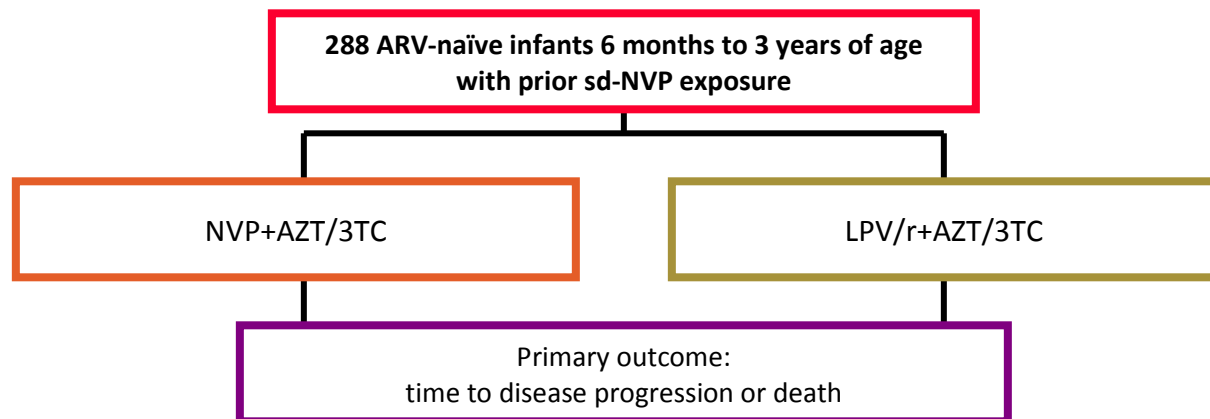
The first published study looking at treatment failure in children previously exposed to sd-NVP was presented by Lockman and colleagues in 2007. They studied the response to NVP-based ARV treatment among thirty mothers and children who were previously part of a trial that involved the administration of either a placebo or sd-NVP for the prevention of MTCT. The results were quite dismal. As seen in Figure 17, there were significantly higher rates of virologic failure among both the mothers and children who previously received sd-NVP in comparison to those previously in the placebo group after NVP-based ARV initiation. By 24 weeks, 77% of the children with previous sd-NVP exposure met viral failure with more than 400copies/mL (Lockman et al. 2007)



**Figure 17.** Time to virologic failure in infants previously exposed to sd-NVP (Lockman et al. 2007).

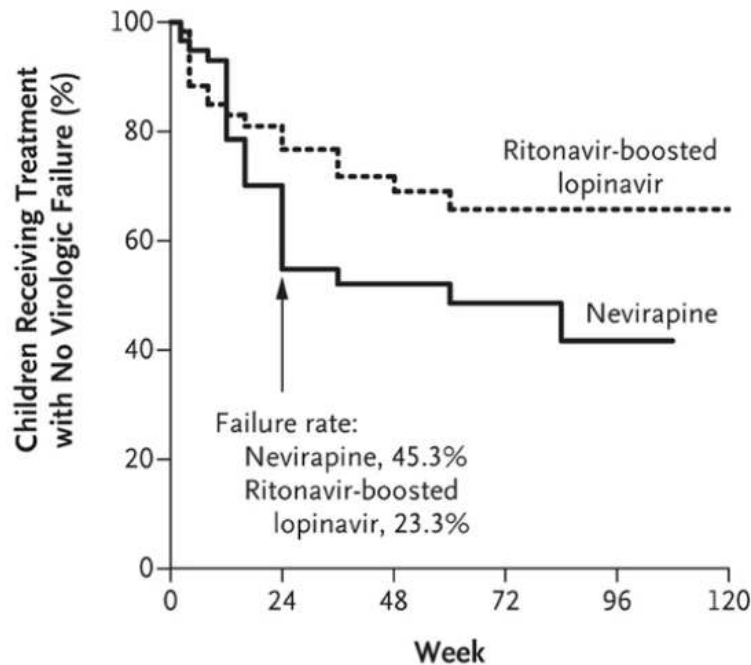
Subsequent ARV treatment after sd-NVP exposure was also studied in the International Maternal Pediatric Adolescent AIDS Clinical Trial (IMPAACT) P1060 trial that was recently been completed by Palumbo and colleagues. This study was designed to

compare NVP versus LPV/r containing regimens given to ART-naïve children six months to three years of age who had prior exposure to sd-NVP.



**Figure 18.** Summary of the P1060 study design (Adapted from Palumbo et al. 2010).

As seen in Figure 18, the children were randomized to NVP+AZT/3TC or to LPV/r+AZT/3TC arms and the primary end point was virologic failure or discontinuation of treatment by week 24. The Data and Safety Monitoring Board (DSMB) recommended closure of this study early due to its early findings. More children in the NVP arm (39.6%) reached a primary viral end point in comparison to the LPV/r arm (21.7%) by 24 weeks. This difference was most distinct in children under the age of one who were put on therapy, with 45.3% in the NVP arm meeting failure compared to 23.3% in the LPV/r arm (Figure 19). Baseline resistance to NVP was also detected at the initiation of treatment in 12% of the children studied and was predictive of treatment failure. For the children in the NVP with detectable resistance prior to therapy, 83% failed in comparison to only 18% in the LVP/r arm with existing resistance (Palumbo et al. 2010). Since NVP is used widely for the prevention of MTCT in lower resource settings, alternative strategies are urgently needed.



**Figure 19.** Time to primary end point of virologic failure or discontinuation of treatment greater in NVP in comparison to LVP/r arm in children under 12 months of age (Palumbo et al. 2010).

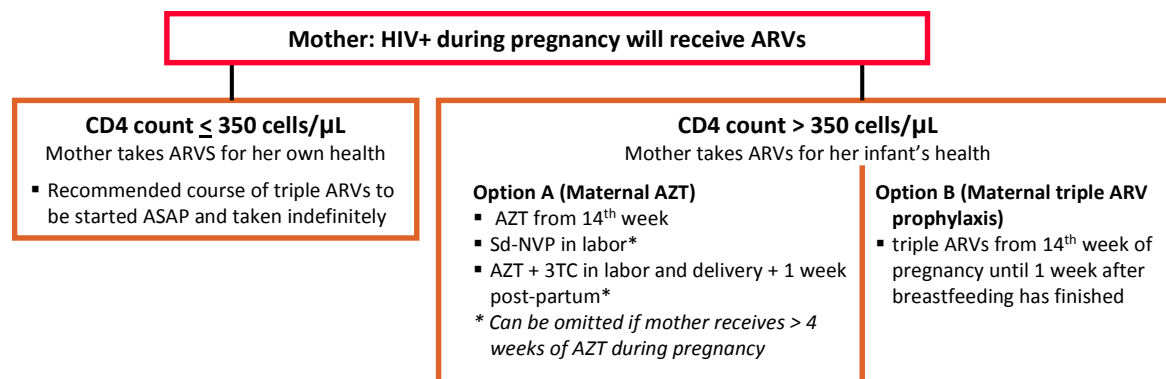
Palumbo et al.'s trial along with the NEVEREST study both showed that there is no association between NNRTI mutations and protease inhibitor therapy (Palumbo et al. 2010, Hunt et al. 2011). New WHO pediatric treatment guidelines now recommend the use of a boosted protease inhibitor such as LPV for therapy in all NVP-exposed children under two years of age (WHO 2010).

## 2010 WORLD HEALTH GUIDELINES FOR THE PREVENTION OF MOTHER-TO-CHILD TRANSMISSION

The new 2010 WHO guidelines for PMTCT are based on two key approaches: lifelong ART and ARV prophylaxis. Lifelong ART is for HIV+ women in need of treatment for their own health, which is also effective in reducing MTCT. ARV prophylaxis prevents MTCT during pregnancy, labor and delivery, and breastfeeding for HIV+ women not in need of

treatment for their own health. These revised guidelines are the first to include recommendations on ARV prophylaxis during breastfeeding where breastfeeding is the most appropriate choice (WHO 2010). This allows PMTCT interventions to continue into the postpartum period.

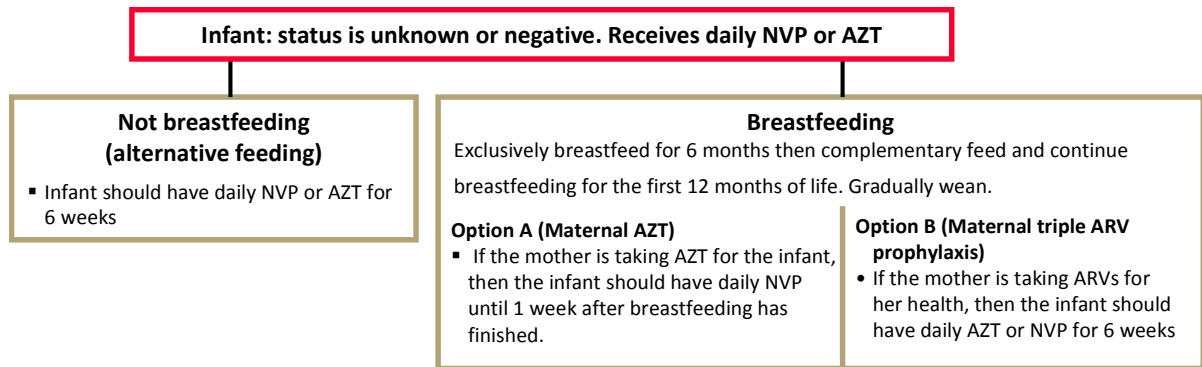
Figures 20 and 21, summarize the new guidelines for both HIV+ pregnant women and their infants. As shown in Figure 20, there is now a focus on maternal therapeutic ARV for women with CD4+ counts less than 350cells/ $\mu$ L. For healthier women with CD4+ counts greater than 350cells/ $\mu$ L, there are two comprehensive options for prophylaxis: A) AZT+ sd-NVP during pregnancy and daily infant NVP during the post natal period or B) maternal ARV prophylaxis during pregnancy and infancy (WHO 2010).



**Figure 20.** Summary of 2010 PMTCT WHO guidelines for HIV+ mothers in lower-resource countries (Adapted from WHO 2010).

Figure 21 illustrates the new guidelines for infants born to HIV+ mothers. All infants should now receive a course of medication which is linked to the drug regimen their mother is taking. If the mother is taking ARVs for her own health, the infant should receive daily NVP for six weeks. If the mother is only taking ARVs for her infant, then the infant should receive daily NVP until one week after breastfeeding is ended. It is recommended that the child breastfeed for the first six months if there is no other safe option. Afterwards, the

mother can supplement breast milk or complementary feed. If the infant is not breastfed, they should receive daily NVP or AZT for six weeks (WHO 2010).



**Figure 21.** Summary of 2010 PMTCT WHO guidelines for infants born to HIV+ mothers in lower-resource countries (Adapted from WHO 2010).

With their recommendation of combination therapy instead of sd-NVP, the new PMTCT WHO guidelines have the potential of bringing transmission rates in lower resource settings to rival the one to two percent transmission rates in higher resource settings. In 2010, Kuhn and colleagues reviewed the potential impact of the new WHO criteria for PMTCT using data from 1,025 HIV-infected women and infants from Zambia. The new criteria of using a CD4+ count below 350cells/ $\mu$ L required initiating therapy in 68% of pregnant women and if fully effective has the possibility of preventing 92% of maternal deaths and 88% of infant infections caused by MTCT (Kuhn et al. 2010).

The problem of pediatric HIV seems so simple to solve. Children need to be identified early on and started on appropriate treatment as seen in the new WHO guidelines. Yet, what about all the children previously exposed to sd-NVP?

## LOPINAVER/RITONAVIR: ANOTHER OPTION FOR CHILDREN PREVIOUSLY EXPOSED TO NEVIRAPINE?

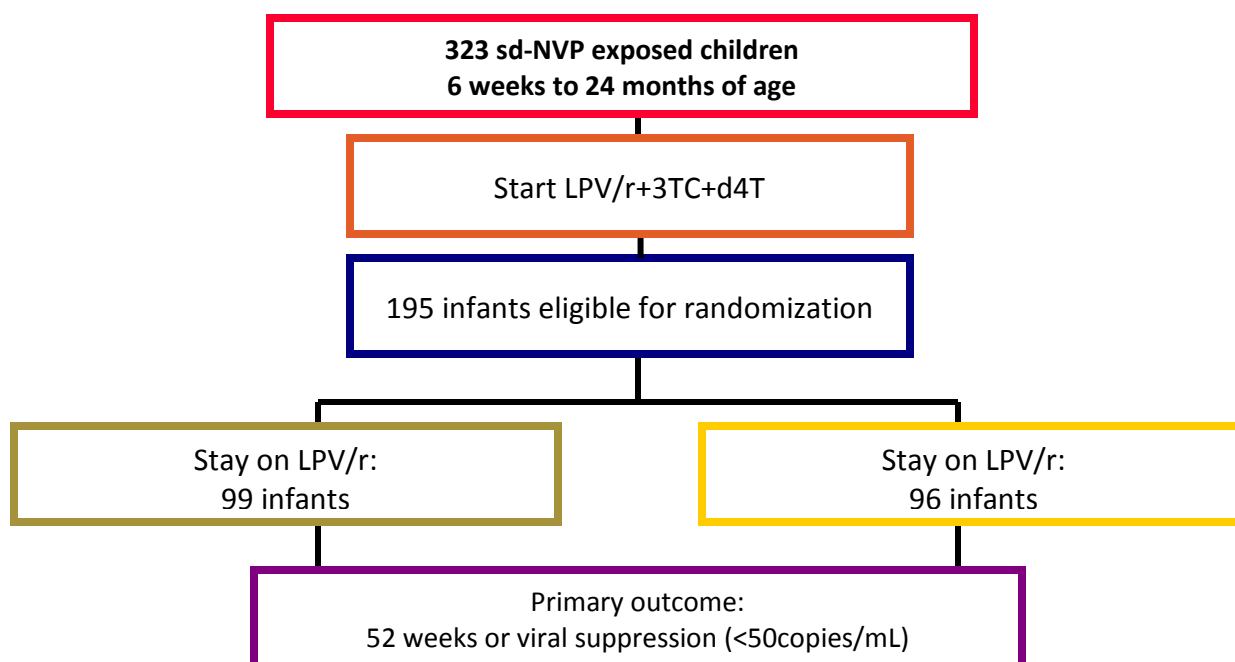
A number of other strategies have looked at whether or not there are other options for children who have been previously exposed to NVP and if there is other options besides NVP for life-long treatment in children. However, as seen in Table 2, there are very few ARV medications currently approved for children in low resource settings (Adams 2010).

**Table 2.** ARV medications approved and available for children in lower resource settings (Adapted from Abrams 2010).

NNRTI	NRTI	Integrase Inhibitor	Protease Inhibitor	CCR5 receptor antagonist	Fixed dose Combination
NVP EFV <sup>1</sup>	ABC <sup>2</sup> DDI D4T 3TC ZDV	-----	LPV/r <sup>2</sup>	-----	NVP+3TC+D4T
<sup>1</sup> Only for children >3 years <sup>2</sup> Not widely available in a majority lower resource countries <sup>3</sup> Limited availability in some lower resource countries					

The global ART scale-up has been anchored on the usage of NNRTI-based regimens due to their low cost including generic and pediatric formulations. Protease inhibitor-based ART is reserved for second-line therapy and has a limited availability and relatively high cost. Lopinavar/ritonavir (LPV/r) is currently the only protease inhibitor option available for young children since dosing and/or formulations of other PIs are not available or approved.

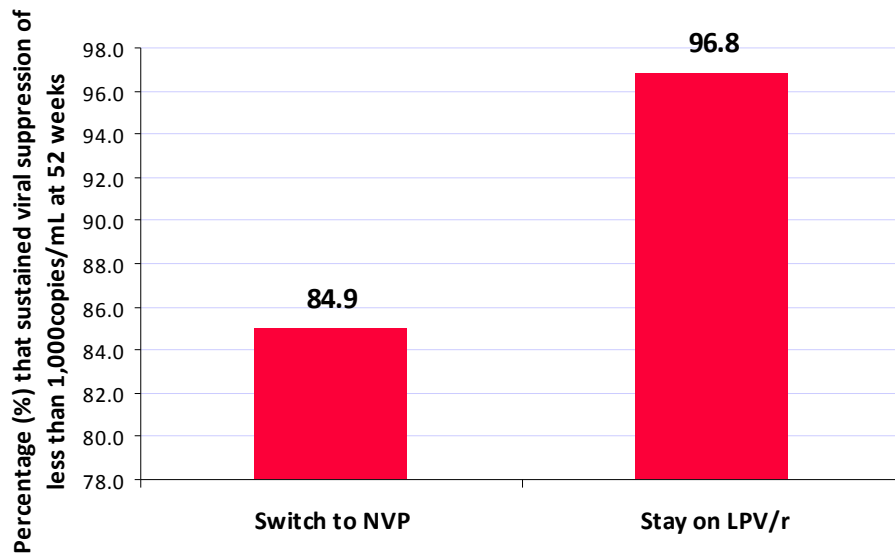
LPV/r was recently used in another arm of the NEVEREST study that looked at sd-NVP exposed children with NNRTI resistance. Their findings indicate that an induction period with full viral suppression after the administration of LPV/r+3TC+d4T (d4T: stavudine) allows for the safe reintroduction of NNRTIs and the ability to stop LPV/r use and reuse it as a second line therapy.



**Figure 22.** Summary of the NEVEREST NVP resistance study design (Adapted from Coovadia et al. 2009).

As shown in the diagram of the study in Figure 22, 323 sd-NVP exposed infants started LPV/r+3TC+d4T treatment and 195 reached viral suppression. The 195 were then randomized to LPV/r or switched to NVP. The primary endpoint was a sustained viral suppression of less than 50copies/mL or 52 weeks. The children who switched to NVP were more likely to fully suppress to less than 50copies/mL. However, upon looking at Figure 23 and the more clinically meaningful sustained viral suppression of less than 1,000copies/mL at 52 weeks, fewer children in the NVP switch group (84.9%) than in the control group (96.8%) were able to maintain suppression (Coovadia et al. 2009). This study illustrates that sd-NVP associated NNRTI mutations prior to the initiation of therapy are directly related to the risk of losing suppression when the infant is switched back to NVP.





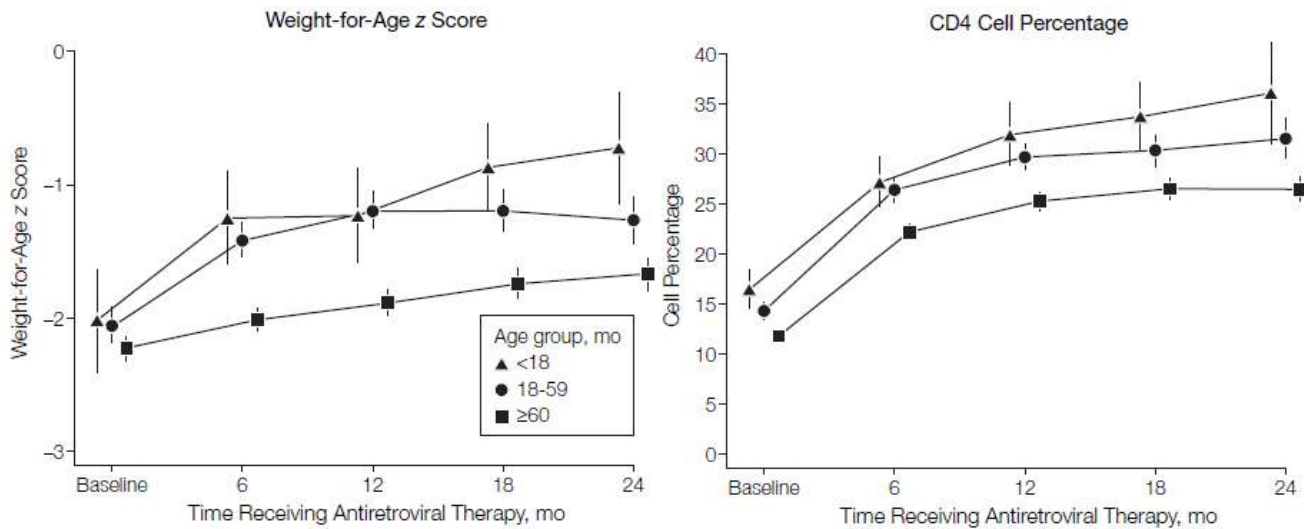
**Figure 23.** NEVEREST NVP resistance study: sustained viral suppression on children in the NVP switch group in comparison to the control group at 52 weeks.

Although LPV/r seems to be definitely an option for PMTCT, there are still many issues that come along with it. Currently, only a liquid form of LPV/r is available for infants and is poorly palatable. LPV/r also requires a cold chain, which is not ideal in transporting the ARV to distant clinics in hot climates served by poorly developed transport networks (Barragan et al. 2008). In addition, LPV/r cannot be used in many HIV+ children who are co-infected with tuberculosis due to its interactions with rifampin, a widely used tuberculosis treatment medication, which jeopardizes viral suppression (Abrams 2010).

So as recent studies support the replacement of sd-NVP with LPV/r, it is important to note that the ARV rollout in most low resource countries has been saving children, especially young children.

## POSITIVE EFFECTS OF ANTIRETROVIRAL THERAPY PROGRAMS IN LOW RESOURCE SETTINGS

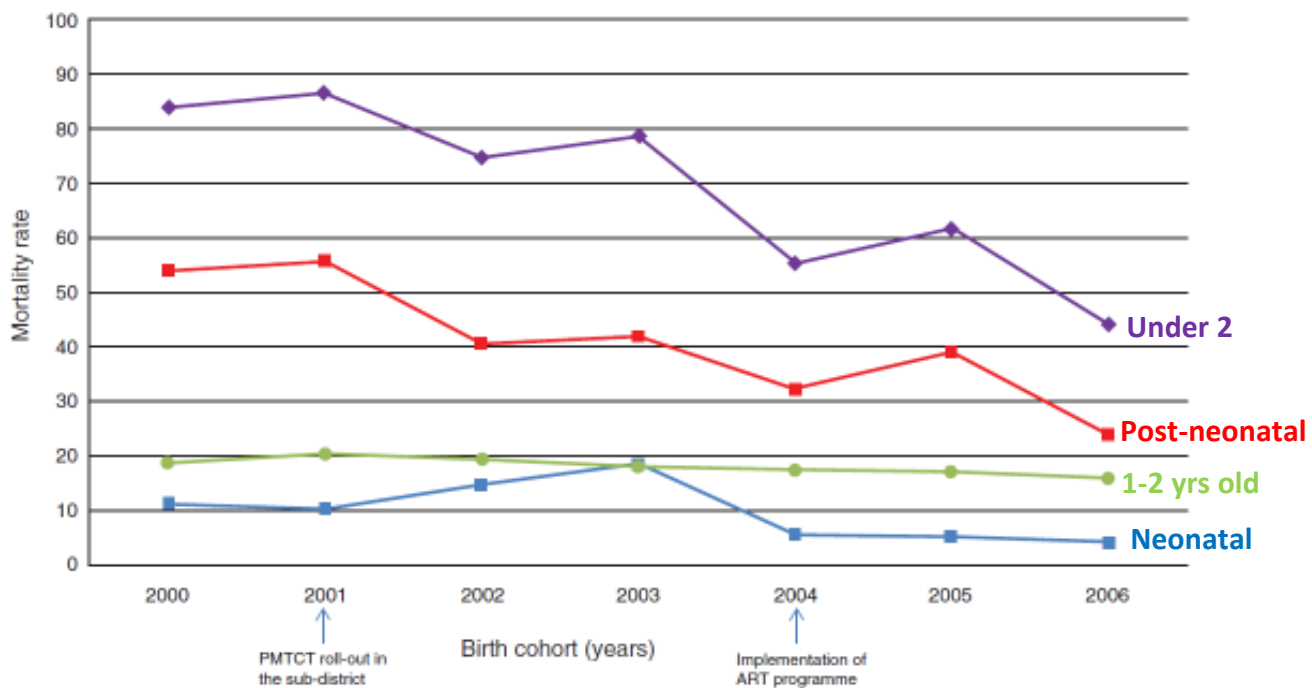
Although it seems like at times PMTCT strategies are failing, there are generally favorable results of ART for children in low resource settings. Even with a modestly functioning PMTCT program, benefits for survival of young children are becoming quickly evident. With more comprehensive treatment coverage, and more efficient PMTCT including AZT in addition to sd-NVP, further significant reductions in early life mortality have been achieved.



**Figure 24.** Children receiving ART have high weight-for-age Z scores and CD4+ percentages, Zambia (Bolton-Moore et al. 2007).

A study done in Zambia demonstrates the excellence of PMTCT programs based on simple regimens in children, especially in young children. In the study, care was provided by clinicians such as nurses and clinical officers in primary health care settings. Children received three-drug ART (AZT+3TC+NVP) if they tested positive for HIV antibodies and showed signs of immunosuppression. In the study, surviving children less than 18 months had high weight-for-age Z scores and high CD4+ counts (Figure 24, Bolton-Moore et al,

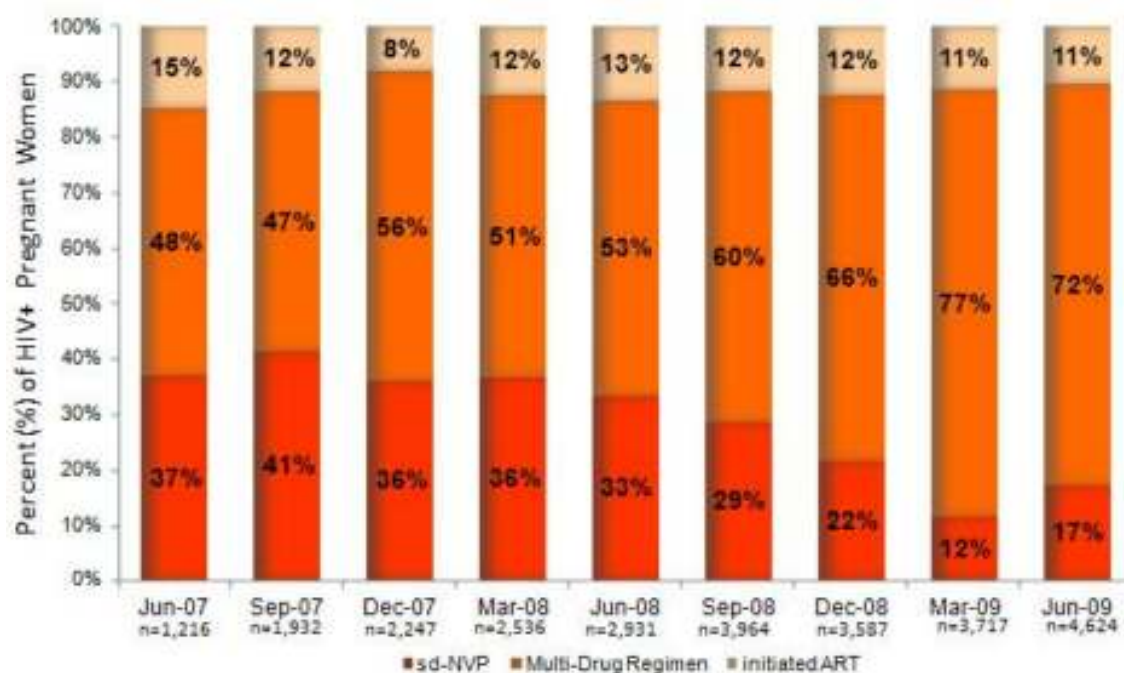
2007). Improvement in weight was more pronounced in the younger children as compared with the older children and the average child experienced more than a doubling of his or her CD4+ cell percentage in the first year of ART. These both show that good clinical outcomes can be obtained by treating children with ART at primary health care facilities using nonphysician clinicians in lower resource settings such as sub-Saharan Africa.



**Figure 25.** Decline in early life mortality with PMTCT ART services in KwaZulu-Natal, South Africa (Adapted from Ndirangua et al. 2010).

When regimens based on AZT plus sd-NVP for the mother and child are not acceptable or feasible, ARV prophylaxis using solely sd-NVP remains a practical regimen. Progress in implementing programs to prevent MTCT based on single-dose maternal and infant NVP or other short course regimens should not be undermined. There has been a large decline in early life mortality of children born to HIV+ mothers in KwaZulu-Natal, South Africa from 2001 to 2006 with the implementation of PMTCT and ART programs based on sd-NVP (Figure 25). Although crude mortality rates in the neonatal and early childhood ages

remained relatively stable, there was a large decline in the postneonatal mortality rates and the numbers of deaths in children under two years of age declined by 49% from 2000 to 2006. With the rollout of a PMTCT program in 2001, child mortality rates declined by 36% followed by a further 20% decline after an HIV treatment program based on sd-NVP was established in 2004 (Ndirangua et al. 2010). These findings confirm that even with a modestly functioning PMTCT and HIV treatment program, children are benefiting.



**Figure 26.** Increased use of more complex ARV regimens in ICAP-supported PMTCT programs 2007-2009 in eight different African countries (ICAP 2010).

The International Center for AIDS Care and Treatment Programs (ICAP) supports HIV care and PMTCT programs in eight different African countries. Figure 26, shows the general trend across all the African sites supported through ICAP over a two year period from 2007 to 2009. It is encouraging to see that there has been a general trend away from sd-NVP to multi-drug regimens, primarily AZT+sd-NVP (ICAP 2010). With more comprehensive

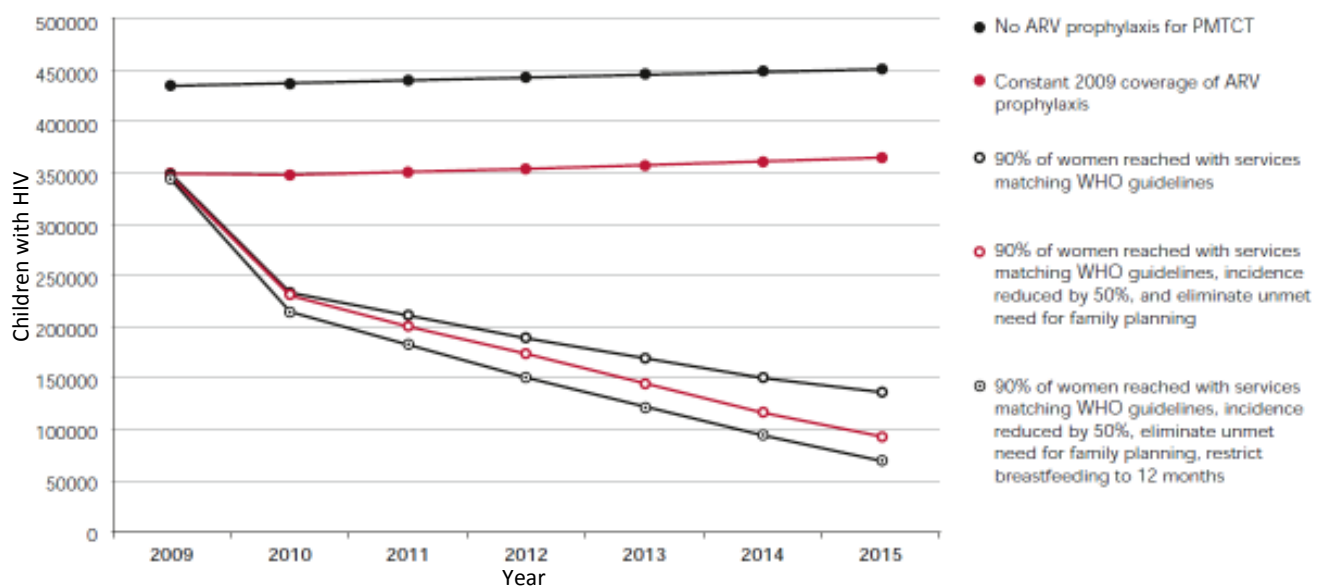
treatment coverage, and more effective PMTCT including combination therapy with AZT in addition to sd-NVP, further significant reductions in child mortality is expected.

Although, progress is being made on the problem of pediatric HIV. The ultimate solution lies in the prevention of mother-to-child transmission and in preventing HIV infection in women. In order for lower resource countries to achieve clinical and immunological outcomes comparable to those seen in higher resource countries such as the United States, the scaling-up of HIV treatment and PMTCT programs needs to be realized.

## **ELIMINATION OF MOTHER-TO-CHILD TRANSMISSION IS POSSIBLE**

Recently in 2009, UNAIDS and partners have called for virtual elimination of HIV transmission from mother-to-child by 2015. Virtual elimination includes the overarching goal of keeping mothers alive, reducing the number of new child HIV infections by 90% between 2009 and 2015, and reducing MTCT to less than 5%. To achieve this, the United Nations recommends the use of their previously mentioned four-pronged approach: (1) primary prevention of HIV infection of women of childbearing age (2) preventing unintended pregnancies among HIV+ women (3) preventing MTCT (4) provided treatment to HIV+ mothers and their families (UNAIDS 2010). In 2009, there were 347,000 new child infections. A 90% reduction would require fewer than 34,700 new infections in 2015 (Mahy et al. 2010). This is an ambitious aim, but also a realistic one that can be achieved with significantly increased implementation of proven strategies. Many lower income countries have already moved significantly towards achieving these goals by achieving at least 80% coverage of services to prevent MTCT, with global coverage reaching 53% (UNAIDS 2010).

Projecting into the future, if current programs were improved so that by 2015 90% of HIV+ pregnant women were provided with ART or effective ARV prophylaxis during pregnancy and throughout breastfeeding as currently recommended by WHO, approximately 1,041,000 new child infections would be averted (Figure 27). This is a 60% reduction in the annual number of new child infections, which is a major step forward but still well below the goal of 90% (Mahy et al. 2010).



**Figure 27.** New HIV infections in children ages 0-14 through MTCT for different scenarios 2009-2015 (Mahy et al. 2010).

Even if there is not exactly a 90% reduction of new child HIV infections by 2015, it is important to note that it is still feasible to one day stop MTCT of HIV. If HIV+ pregnant women and their children have timely access to quality ARVs—for their own health or as prophylaxis to stop HIV transmission during pregnancy, delivery, and breastfeeding—it is possible to stop new HIV infections among children and keep their mothers alive. This is where the importance of moving rapidly to the new, more effective ARV interventions recommended in the 2010 WHO guidelines comes into play. Using the new WHO guidelines

for ARVs, MTCT can be virtually eliminated and together with a comprehensive approach to reduce new infections in women and meet family planning needs, rapid progress can be made towards virtual elimination.

## **CONCLUSIONS**

Ultimately, a large number of mothers and children continue to receive sub-optimal single-dose nevirapine as their main HIV prophylaxis. This must be phased out not only in accordance with the 2010 WHO guidelines, but in order to reach the new target of virtual elimination of mother-to-child transmission.

It is clear that the use of sd-NVP places both mother and child at risk for acquiring NNRTI resistance mutations which creates challenges to achieving successful subsequent treatment. NNRTI resistance in infants exposed to sd-NVP compromises the response to subsequent NNRTI-based treatment and can jeopardize simpler first-line treatment, necessitating the use of second-line regimens containing drugs that are costlier and more difficult to administer. This is particularly worrisome in infants initiating lifelong therapy.

LPV/r-based ART is the preferred regimen for HIV-infected infants with prior NVP exposure for PMTCT prophylaxis. However, there are multiple issues associated with LPV/r and there are few options currently available for children who do not tolerate or fail first-line LPV/r-based ART.

On the other hand, a program to prevent MTCT that cannot deliver or ensure adherence to a more complex prophylaxis regimen is less effective than a program that implements the simpler sd-NVP regimen even though the more complex regimen may have

shown greater efficacy in clinical trials. Although sd-NVP may not be the most optimal regimen, it is important to realize that there are still more than a thousand new pediatric infections occurring each day, primarily due to mother-to-child transmission. Sd-NVP will always outweigh the high risk for death and disease progression associated with no interventions.

In the end, alternative approaches and new ARVs are urgently needed to ensure safe and successful lifelong ART for infants and children with HIV infection. For it is the scale-up of more effective ARV interventions, together with a comprehensive approach to reduce new infections in pregnant women and meet family planning needs, that creates rapid progress towards virtual elimination of mother-to-child transmission of HIV.





## **ACKNOWLEDGEMENTS**

I would like to thank my primary reader, Professor James Higdon of W.M. Keck Science Department, for his encouragement and continual support. I am also appreciative of my secondary reader, Professor Karl Haushalter of Harvey Mudd College, whose HIV-AIDS: Science, Society, and Service course gave me an awareness of the subject and whose enthusiasm and knowledge was invaluable during the development of my thesis. I would also like to thank parents, Tim and Jeanette Harmon, for their unyielding love and encouragement.



## LITERATURE CITED

### Journal Articles

Andiman, W., et al. "The Mode of Delivery and the Risk of Vertical Transmission of Human Immunodeficiency Virus Type 1 - A Meta-Analysis of 15 Prospective Cohort Studies." *New England Journal of Medicine* 340.13 (1999): 977-87. Print.

Arrivé, Elise, et al. "Prevalence of Resistance to Nevirapine in Mothers and Children After Single-Dose Exposure to Prevent Vertical Transmission of HIV-1: A Meta-Analysis." *International journal of epidemiology* 36.5 (2007): 1009-21. Print.

Barragan, Patricia, and Daniel Podzamczar. "Lopinavir/ritonavir: A Protease Inhibitor for HIV-1 Treatment." *Expert opinion on pharmacotherapy* 9.13 (2008): 2363-75. Print.

Bolton-Moore, Carolyn, et al. "Clinical Outcomes and CD4 Cell Response in Children Receiving Antiretroviral Therapy at Primary Health Care Facilities in Zambia." *Jama-Journal of the American Medical Association* 298.16 (2007): 1888-99. Print.

Campiani, G., et al. "Non-Nucleoside HIV-1 Reverse Transcriptase (RT) Inhibitors: Past, Present, and Future Perspectives." *Current pharmaceutical design* 8.8 (2002): 615-57. Print.

Church, Jessica D., et al. "Analysis of Nevirapine (NVP) Resistance in Ugandan Infants Who were HIV Infected Despite Receiving Single-Dose (SD) NVP Versus SD NVP Plus Daily NVP Up to 6 Weeks of Age to Prevent HIV Vertical Transmission." *Journal of Infectious Diseases* 198.7 (2008): 1075-82. Print.

Connor, E. M., et al. "Reduction of Maternal-Infant Transmission of Human-Immunodeficiency-Virus Type-1 with Zidovudine Treatment." *New England Journal of Medicine* 331.18 (1994): 1173-80. Print.

Conway, B., et al. "Development of Drug Resistance in Patients Receiving Combinations of Zidovudine, Didanosine and Nevirapine." *Aids* 15.10 (2001): 1269-74. Print.

Cressey, T. R., et al. "Persistence of Nevirapine Exposure during the Postpartum Period After Intrapartum Single-Dose Nevirapine in Addition to Zidovudine Prophylaxis for the

- Prevention of Mother-to-Child Transmission of HIV-1." *Aids-Journal of Acquired Immune Deficiency Syndromes* 38.3 (2005): 283-8. Print.
- Cunningham, C. K., et al. "Development of Resistance Mutations in Women Receiving Standard Antiretroviral Therapy Who Received Intrapartum Nevirapine to Prevent Perinatal Human Immunodeficiency Virus Type 1 Transmission: A Substudy of Pediatric AIDS Clinical Trials Group Protocol 316." *Journal of Infectious Diseases* 186.2 (2002): 181-8. Print.
- D'Aquila, R. T., et al. "Nevirapine, Zidovudine, and Didanosine Compared with Zidovudine and Didanosine in Patients with HIV-1 Infection - A Randomized, Double-Blind, Placebo-Controlled Trial." *Annals of Internal Medicine* 124.12 (1996): 1019-30. Print.
- De Cock, KM, et al. "Prevention of Mother-to-Child HIV Transmission in Resource-Poor Countries - Translating Research into Policy and Practice." *Jama-Journal of the American Medical Association* 283.9 (2000): 1175-82. Print.
- Deeks, S. G. "Nonnucleoside Reverse Transcriptase Inhibitor Resistance." *Journal of acquired immune deficiency syndromes* 26 (2001): S25-33. Print.
- Duerr, A., et al. "Integrating Family Planning and Prevention of Mother-to-Child HIV Transmission in Resource-Limited Settings." *Lancet* 366.9481 (2005): 261-3. Print.
- Dunkle, Kristin L., et al. "New Heterosexually Transmitted HIV Infections in Married Or Cohabiting Couples in Urban Zambia and Rwanda: An Analysis of Survey and Clinical Data." *Lancet* 371.9631 (2008): 2183-91. Print.
- Eshleman, S. H., et al. "Selection and Fading of Resistance Mutations in Women and Infants Receiving Nevirapine to Prevent HIV-1 Vertical Transmission (HIVNET 012)." *Aids* 15.15 (2001): 1951-7. Print.
- Flys, Tamara S., et al. "Nevirapine Resistance in Women and Infants After First Versus Repeated use of Single-Dose Nevirapine for Prevention of HIV-1 Vertical Transmission." *Journal of Infectious Diseases* 198.4 (2008): 465-9. Print.
- Garcia, P. M., et al. "Maternal Levels of Plasma Human Immunodeficiency Virus Type 1 RNA and the Risk of Perinatal Transmission." *New England Journal of Medicine* 341.6 (1999): 394-402. Print.
- Guay, L. A., et al. "Intrapartum and Neonatal Single-Dose Nevirapine Compared with Zidovudine for Prevention of Mother-to-Child Transmission of HIV-1 in Kampala, Uganda: HIVMET 012 Randomised Trial." *Lancet* 354.9181 (1999): 795-802. Print.

- Hammer, Scott M., et al. "Antiretroviral Treatment of Adult HIV Infection - 2008 Recommendations of the International AIDS Society USA Panel." *Jama-Journal of the American Medical Association* 300.5 (2008): 555-70. Print.
- Hunt, Gillian M., et al. "HIV-1 Drug Resistance at Antiretroviral Treatment Initiation in Children Previously Exposed to Single-Dose Nevirapine." *Aids* 25.12 (2011): 1461-9. Print.
- Jasseron, Carine, et al. "Prevention of Mother-to-Child HIV Transmission: Similar Access for Sub-Sahara African Immigrants and for French Women?" *Aids* 22.12 (2008): 1503-11. Print.
- Katz, Mitchell H. "HIV/AIDS 2010 Better Drugs, Better Technologies, and Lingering Problems." *Archives of Internal Medicine* 170.1 (2010): 6-8. Print.
- Lee, E. J., et al. "Breast-Milk Shedding of Drug-Resistant HIV-1 Subtype C in Women Exposed to Single-Dose Nevirapine." *Journal of Infectious Diseases* 192.7 (2005): 1260-4. Print.
- Lockman, Shahin, et al. "Response to Antiretroviral Therapy After a Single, Peripartum Dose of Nevirapine." *New England Journal of Medicine* 356.2 (2007): 135-47. Print.
- Mahy, Mary, et al. "What Will it Take to Achieve Virtual Elimination of Mother-to-Child Transmission of HIV? an Assessment of Current Progress and Future Needs." *Sexually transmitted infections* 86 (2010): 148-55. Print.
- Marinda, Edmore, et al. "Child Mortality According to Maternal and Infant HIV Status in Zimbabwe." *Pediatric Infectious Disease Journal* 26.6 (2007): 519-26. Print.
- Marseille, E., et al. "Cost Effectiveness of Single-Dose Nevirapine Regimen for Mothers and Babies to Decrease Vertical HIV-1 Transmission in Sub-Saharan Africa." *Lancet* 354.9181 (1999): 803-9. Print.
- Menéndez-Arias, L. M., Betancor, G., & Matamoros, T. (2011). HIV-1 reverse transcriptase connection subdomain mutations involved in resistance to approved non-nucleoside inhibitors. *Antiviral Research*, 92(2), 139–149. Print.
- Mermin, Jonathan, et al. "Mortality in HIV-Infected Ugandan Adults Receiving Antiretroviral Treatment and Survival of their HIV-Uninfected Children: A Prospective Cohort Study." *Lancet* 371.9614 (2008): 752-9. Print.
- Mirochnick, Mark, et al. "Antiretroviral Concentrations in Breast-Feeding Infants of Mothers Receiving Highly Active Antiretroviral Therapy." *Antimicrobial Agents and Chemotherapy* 53.3 (2009): 1170-6. Print.

- Mofenson, L. M., and P. Munderi. "Safety of Antiretroviral Prophylaxis of Perinatal Transmission for HIV-Infected Pregnant Women and their Infants." *Journal of acquired immune deficiency syndromes* 30.2 (2002): 200-15. Print.
- Moorthy, Anitha, et al. "Nevirapine Resistance and Breast-Milk HIV Transmission: Effects of Single and Extended-Dose Nevirapine Prophylaxis in Subtype C HIV-Infected Infants." *Plos One* 4.1 (2009): e4096. Print.
- Ndirangu, James, et al. "Decline in Early Life Mortality in a High HIV Prevalence Rural Area of South Africa: Evidence of HIV Prevention Or Treatment Impact?" *Aids* 24.4 (2010): 593-602. Print.
- Newell, M. L., et al. "Mortality of Infected and Uninfected Infants Born to HIV-Infected Mothers in Africa: A Pooled Analysis." *Lancet* 364.9441 (2004): 1236-43. Print.
- Palumbo, Paul, et al. "Antiretroviral Treatment for Children with Peripartum Nevirapine Exposure." *New England Journal of Medicine* 363.16 (2010): 1510-20. Print.
- Patel, S. S., and P. Benfield. "Nevirapine." *Clinical Immunotherapeutics* 6.4 (1996): 307-17. Print.
- Ren, J., et al. "Structure of HIV-2 Reverse Transcriptase at 2.35-Angstrom Resolution and the Mechanism of Resistance to Non-Nucleoside Inhibitors." *Proceedings of the National Academy of Sciences of the United States of America* 99.22 (2002): 14410-5. Print.
- Shearer, W. T., et al. "Viral Load and Disease Progression in Infants Infected with Human Immunodeficiency Virus Type 1." *New England Journal of Medicine* 336.19 (1997): 1337-42. Print.
- Thomas, Timothy K., et al. "Triple-Antiretroviral Prophylaxis to Prevent Mother-to-Child HIV Transmission through Breastfeeding-the Kisumu Breastfeeding Study, Kenya: A Clinical Trial." *Plos Medicine* 8.3 (2011): e1001015. Print.
- Van Dyke, Russell B., et al. "Toxicities Associated with Dual Nucleoside Reverse-Transcriptase Inhibitor Regimens in HIV-Infected Children." *Journal of Infectious Diseases* 198.11 (2008): 1599-608. Print.
- Violari, Avy, et al. "Early Antiretroviral Therapy and Mortality among HIV-Infected Infants." *New England Journal of Medicine* 359.21 (2008): 2233-44. Print.
- Zeh, Clement, et al. "HIV-1 Drug Resistance Emergence among Breastfeeding Infants Born to HIV-Infected Mothers during a Single-Arm Trial of Triple-Antiretroviral Prophylaxis for Prevention of Mother-to-Child Transmission: A Secondary Analysis." *Plos Medicine* 8.3 (2011): e1000430. Print.

## Reports

ICAP (International Center for AIDS Care and Treatment Programs). "Improving Retention, Adherence and Psychosocial Support within PMTCT Services: A Toolkit for Health Workers." Columbia University: 2010. Print.

International AIDS Society-USA. "Update on the Drug Resistance Mutations in HIV-1: December 2010." *Topics in HIV Medicine* 18.5 (2010). Print.

Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission. Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-1-Infected Women for Maternal Health and Interventions to Reduce Perinatal HIV Transmission in the United States. September 14, 2011; pp 1-207.

UNAIDS (Joint United Nations Programme on HIV/AIDS). *AIDS Epidemic Update 2009*, 2009. Print.

UNAIDS (Joint United Nations Programme on HIV/AIDS). *Global Report: UNAIDS Report on the Global AIDS Epidemic 2010*, 2010. Print.

UNAIDS (Joint United Nations Programme on HIV/AIDS). *2008 Report on the Global AIDS Epidemic*. Geneva: World Health Organization, 2008. Print.

UNICEF (United Nations Children's Fund). *Children and Aids: Fourth Stocktaking Report, 2009*. New York: UNICEF, 2009. Print.

World Health Organization (WHO). *Antiretroviral Drugs for Treating Pregnant Women and Preventing HIV Infection in Infants: Recommendations for a Public Health Approach, 2010 version*. Geneva: World Health Organization, Department of HIV/AIDS, 2010. Print.

## Books

Levy, Jay A. *HIV and the Pathogenesis of Aids*. Washington, D.C: ASM Press, 2007. Print.

Stine, Gerald J. *Aids Update 2010: An Annual Overview of Acquired Immune Deficiency Syndrome*. New York, N.Y: McGraw Hill, 2010. Print.



Weeks, Benjamin S, and I E. Alcamo. *Aids: The Biological Basis*. Sudbury, Mass: Jones and Bartlett Publishers, 2010. Print.

#### Web documents

Abrams, Elaine. "Putting the "C" into MTCT—Saving Kids." Lecture. 17th Conference on Retroviruses and Opportunistic Infections. San Francisco. 17 Feb. 2010. *CROI*. Web.

Cooavadia, A. et al. "Randomized clinical trial of switching to nevirapine-based therapy for infected children exposed to nevirapine prophylaxis." *International Aids Society*, 2009. Web.

*Review of the HIVNET 012 Perinatal HIV Prevention Study*. Washington, D.C: National Academies Press, 2005. Web.



